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(54) Title: PLASMID VECTORS FOR TRANSFORMATION OF FILAMENTOUS FUNGI

(57) Abstract: The invention relates to novel plasmid vectors for transformation of filamentous fungi and to a method of modifiying the genome of filamentous fungi based on these vectors. The invention furthermore relates to the modification of a specific gene via the process of homologous recombination, to recombinant expression of foreign genes in filamentous fungi and to new selection markers for detecting succesfull transfer of the target gene in filamentous fungi.

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Plasmid vectors for transformation of filamentous fungi

Description

The invention relates to novel plasmid vectors for transformation of filamentous fungi and to a method of modifiying the genome of filamentous fungi based on these vectors. The invention furthermore relates to the modification of a specific gene via the process of homologous recombination, to recombinant expression of foreign genes in filamentous fungi and to new selection markers for detecting the successful transfer of the target gene in filamentous fungi.

One method currently used for transformation of filamentous fungi is random mutagenesis based on transposons insertion, a method also known for plant transformation (WO 01/38509). This method allows the genomes of several species such as Magnaporthe grisea to be studied (for examples WO 00/55346; WO 00/56902). However, this strategy requires a big effort in terms of bioinformatic tools and molecular biology to localise precisely the insertion in the genome.

Alternatively, known transformation methods are based on targeted integration. Targeted transformation of fungi can be carried out either by offering a knock-out cassette with a marker-gene flanked by two homologous sequences (Aronson et al, 1994, Mol. Gen. Genet. 242: 490-494; Royer et al, 1999, Fungal Genetics and Biology 28: 68-78; Schaefer, 2001, Current Opinion in Plant Biology 4: 143-150) or by quoting a plasmid with the marker gene in the neighburship of a homologous sequence (Shortle et al.,1982, Science 217: 371-373; Bird and Bradshaw, 1997, Mol Gen Genet. 255: 219-225; Feng et al., 2001, Infection and Immunity 69 (3): 1781-1794; Schaefer, 2001, Current Opinion in Plant Biology 4: 143-150). Both procedures are in principle attractive methods to study the gene function, but they have the disadvantage of a high frequency of integration at ectopic sites by illegitimate recombination. The gene targeting efficiency (gene targeting / gene targeting + illegitimate recombination) is 95% for S. cerevisiae, 10-90% for S. pombe, 5-75% for Aspergillus nidulans and 1-30% for Neuropsora crassa using a size of homology of 2-9 Kb (Schaefer, 2001, Current Opinion in Plant Biology 4: 143-150). Especially for filamentous fungi this side effect is quite high, if conventional plasmid vectors are used.

In addition, the efficiency of the gene targeting increases if the length of homologous recombination region is increased (Schaefer, 2001, Current Opinion in Plant Biology 4: 143-150). Thus, plasmid vectors currently used comprise gene fragments of the gene to be knocked out of a size of at least 2000bp as indicated above. The overall size of these plasmid vectors is at least 8000bp (P. J. Punt et. al., 1992, Methods in Enzymology, vol.216, pp 447-457; ; Schaefer, 2001, Current Opinion in Plant Biology 4: 143-150). Since transformation efficiency decreases with the increase of the plasmid vector size, transformation efficiency is unsatisfactury giving rise to long times until positive clones can be identified. This is an obstacle especially to large scale genomic analysis projects or recombinant expression.

Furthermore, currently used plasmid vectors contain many unique restriction sites, causing difficulties in construction of the knock-out (KO-) plasmids and the transformation process. The efficiency of homologous recombination is improved when the KO-plasmid is digested with a restriction enzyme presenting a unique site in the middle of a DNA fragment homologous to the targeted gene. The presence of high amounts of restriction sites especially unique ones in the plasmid backbone decreases the chance of finding a natural restriction site in the appropriate location of the targeted DNA fragment. This problem is usually overcome by modification of the targeted DNA fragment requiring several cloning steps and additional manipulation in terms of molecular biology, which is a disadvantageous and time consuming methology.

Integration of recombinant gen by homolgous recombination in fungi is also a tool to identify gene functions for essential genes: the biochemical characterization of an essential gene cannot be studied by classical knock-out strategy since the mutants carrying a disruption of such a gene are not viable. One way to overexpress such a gene overcomes the problem when a typical phenotype can be assigned to the mutant that overexpresses the gene. Another approach can be to regulate the gene expression by an inducible promoter sequence so that the gene could be expressed or repressed when needed and consequently permits viable mutants to be isolated. As mentioned above, these approaches require at least several thousand bp of the nucleic acid sequence to be studied that need to be integrated in the genome of the fungi together with a plasmid vector comprising the different parts of the nucleic acid sequence. In addition, if the recombinant DNA is integrated at an ectopic site, the identification of the mutant strains becomes more complicated and the position of the integration in the genome may influence the level of expression of the recombinant protein. Taking the aforesaid into consideration,

currently existing plasmid vectors currently used for transformation of filamentous fungi exhibit a lot of disadvantages and are neither suitable for large scale analysis e.g. in functional genomic studies nor convenient for recombinant expression in a filamentous fungi. Additionally, there is a constant need for new selection markers facilitating the selection process.

Thus, object of the present invention was to develop tools for targeted transformation of filamentous fungi that overcome the disadvantages of the state of the art like plasmid vectors suitable for functional genomic studies and recombinant expression and new selection markers.

We have found that the object of the invention is achieved by construction of a plasmid vector for targeted transformation of filamentous fungi comprising

a) an origin of replication for a host organism not originating from the filamentous fungi to be transformed;

b) a selection marker for a host organism not originating from the filamentous fungi to be transformed;

c) a promotor facilitating recombinant expression in filamentous fungi that is functionally linked to the coding region of the hygromycin resistance gene which is functionally linked to a terminator which facilitates transcription termination in filamentous fungi;

wherein the overall size of the elements a), b) and c) does not exceed 4500 bp; and

d) a nucleic acid sequence, which is homologous to nucleic acid sequences of the filamentous fungi to be transformed and makes homologous recombination in the filamentous fungi to be transformed possible.

The term overall size of the elements a), b) and c) designates the combination of the essential elements of the expression vector without the nucleic acid sequence d).

The overall size of the elements a), b) and c) does not exceed 4500 bp, preferaby 4100 bp, more preferably 3700 bp.

In addition to the nucleic acid elements a), b), c) and d), the plasmid vector optionally comprises a cloning site containing rare restriction sites or a TA-cloning site by which further nu-

cleic acid sequences can be cloned easily into the plasmid vector. A TA-cloning site comprises thymidine residues linked onto the 3'-ends of linearized plasmid DNA, which would allow some annealing to occur between the vector and the A-tailed PCR product to be ligated. This process is called TA cloning. Preferably, the vector is modified in such a way that there are only few unique restriction sites left enabling the digestion by commercially available restriction enzymes of the homologous sequence of the targeted gene prior to the transformation.

Filamentous fungi that can be transformed with the vectors of the present invention are non-phytopathogenic filamentous fungi e.g. Neurospora species like Neurospora crassa and phytopathogenic filamentous fungi the phytopathogenic filamentous fungi being preferred. Examples of other non-pythopathogenic filamentous fungi are Aspergillus species such as Aspergillus parasiticus, Aspergillus nidulans, Aspergillus niger and Wagiella such as Wangiella dermatidis. Preferred phytopathogenic filamentous fungi are selected from the group consisting of the genera Alternaria, Podosphaera, Sclerotinia, Physalospora, Botrytis, Corynespora; Colletotrichum; Diplocarpon; Elsinoe; Diaporthe; Sphaerotheca; Cinula, Cercospora; Erysiphe; Sphaerotheca; Leveillula; Mycosphaerella; Phyllactinia; Gloesporium; Gymnosporangium, Leptotthrydium, Podosphaera; Gloedes; Cladosporium; Phomopsis; Phytopora; Phytophthora; Erysiphe; Fusarium; Verticillium; Glomerella; Drechslera; Bipolaris; Personospora; Phaeoisariopsis; Spaceloma; Pseudocercosporella; Pseudoperonospora; Puccinia; Typhula; Pyricularia; Rhizoctonia; Stachosporium; Uncinula; Ustilago; Gaeumannomyces and Fusarium, more preferred from the group consisting of the genera and species Alternaria, Podosphaera, Sclerotinia, Physalospora such as Physalospora canker, Botrytis species such as Botrytis cinerea, Corynespora such as Corynespora melonis; Colletotrichum; Diplocarpon such as Diplocarpon rosae; Elsinoe such as Elsinoe fawcetti, Diaporthe such as Diaporthe citri; Sphaerotheca; Cinula such as Cinula neccata, Cercospora; Erysiphe such as Erysiphe cichoracearum and Erysiphe graminis; Sphaerotheca such as Sphaerotheca fuliginea; Leveillula such as Leveillula taurica; Mycosphaerella; Phyllactinia such as Phyllactinia kakicola; Gloesporium such as Gloesporium kaki; Gymnosporangium such as Gymnosporangium yamadae, Leptotthrydium such as Leptotthrydium pomi, Podosphaera such as Podosphaera leucotricha; Gloedes such as Gloedes pomigena; Cladosporium such as Cladosporium carpophilum; Phomopsis; Phytopora; Phytophthora such as Phytophthora infestans; Verticillium; Glomerella such as Glomerella cingulata; Drechslera; Bipolaris; Personospora; Phaeoisariopsis such as Phaeoisariopsis vitis; Spaceloma such as Spaceloma ampelina; Pseudocercosporella such as Pseudocercosporella herpotrichoides;

Pseudoperonospora; Puccinia; Typhula; Pyricularia such as Pyricularia oryzae; Rhizoctonia; Stachosporium such as Stachosporium nodorum; Uncinula such as Uncinula necator; Ustilago; Gaeumannomyces species such as Gaeumannomyces graminis and Fusarium such as Fusarium dimerium, Fusarium merismoides, Fusarium lateritium, Fusarium decemcellulare, Fusarium poae, Fusarium tricinctum, Fusarium sporotrichioides, Fusarium chlamydosporum, Fusarium moniliforme, Fusarium proliferatum, Fusarium anthophilum, Fusarium subglutinans, Fusarium nygamai, Fusarium oxysporum, Fusarium solani, Fusarium culmorum, Fusarium sambucinum, Fusarium crookwellense, Fusarium avenaceum ssp. avenaceum, Fusarium avenaceum ssp. aywerte, Fusarium avenaceum ssp. nurragi, Fusarium hetrosporum, Fusarium acuminatum ssp. acuminatum, Fusarium acuminatum ssp. armeniacum, Fusarium longipes, Fusarium compactum, Fusarium equiseti, Fusarium scripi, Fusarium polyphialidicum, Fusarium semitectum and Fusarium beomiforme and especially preferred from the genera Fusarium such as Fusarium graminearum, most preferred from the group consisting of the genera and species Fusarium, Fusarium dimerium, Fusarium merismoides, Fusarium lateritium, Fusarium decemcellulare, Fusarium poae, Fusarium tricinctum, Fusarium sporotrichioides, Fusarium chlamydosporum, Fusarium moniliforme, Fusarium proliferatum, Fusarium anthophilum, Fusarium subglutinans, Fusarium nygamai, Fusarium oxysporum, Fusarium solani, Fusarium culmorum, Fusarium sambucinum, Fusarium crookwellense, Fusarium avenaceum ssp. avenaceum, Fusarium avenaceum ssp. aywerte, Fusarium avenaceum ssp. nurragi, Fusarium hetrosporum, Fusarium acuminatum ssp. acuminatum, Fusarium acuminatum ssp. armeniacum, Fusarium longipes, Fusarium compactum, Fusarium equiseti, Fusarium scripi, Fusarium polyphialidicum, Fusarium semitectum and Fusarium beomiforme wherein Fusarium graminearum is most preferred.

The host organism in which the origin of replication a) is functionally active essentially serves to construct and propagate the plasmid vector of the invention. The host organism must be genetically different from the filamentous fungi to be transformed, since replication of the plasmid vector should not take place in the filamentous fungi to be transformed but is desired in the host organism, due to the use of the origin of replication a). Host organisms which may be used are all common microorganisms which can easily be manipulated by genetic engineering. Preferred host organisms are Gram-negative bacteria such as the genera Escherichia and Salmonella e.g. Escherichia coli and Salmonella thyplimurium or Gram-positive bacteria such as the genera Bacillus and Streptomyces, e.g. Bacillus subtilis and Streptomyces nidulans. Particularly preferred are gram-negative bacteria such as

Escherichia, e.g. Escherichia coli.

Preferred origins of replication (ori) are the col E1 ori, the f1 ori.

The term "selection marker for a host organism" set forth in b) means a gene or the expression product of the gene. Preferred meanings are genes whose expression causes resistance of the host organism to antibiotics, by preference resistance to kanamycin, chloramphenicol, tetracycline, zeocin or ampicillin, and particularly preferred ampicillin and kanamycin.

In a preferred embodiment, the element a) of the plasmid vector according to the invention comprises a col E1 origin of replication and the ampicillin resistance gene as selection marker for the host organism.

The element c) is hereinbelow termed "hygromycin cassette". The coding region of the hygromycin resistance gene (hereinbelow termed "hygromycin gene") is known by the skilled artisan (Gritz L. and Davies J. ,1983, Gene 25, 179-188, Kaster, K.R., Burgett S.G. and Ingolia T.D., 1984, Curr. Genet. 8,353-358) and has a length of 1026bp.

Examples of suitable promotors to which the coding region of the hygromycin gene is functionally linked, are the GPD-1-, PX6-, TEF-, CUP1-, PGK-, GAP1-, TPI, PHO5-, AOX1, GAL10/CYC-1, CYC1, OliC-, ADH-, TDH-, Kex2-, MFα-, or the NMT-promotor (Degryse et al., Yeast 1995 Jun 15; 11(7):629-40; Romanos et al. Yeast 1992 Jun;8(6):423-88; Benito et al. Eur. J. Plant Pathol. 104, 207-220 (1998); Cregg et al. Biotechnology (N Y) 1993 Aug;11(8):905-10; Luo X., Gene 1995 Sep 22;163(1):127-31; Nacken et al., Gene 1996 Oct 10;175(1-2):253-60; Turgeon et al., Mol Cell Biol 1987 Sep;7(9):3297-305), preferably the CYC1-, ADH-, TDH-, Kex2-, GPD-1-, PX6, TEF-, CUP1-, PGK-, GAP1-, TPI, PHO5- or AOX1-promotor, more preferably the GPD-1-, PX6, TEF- or the CUP1-promotor, most preferably the GPD1 or the TEF-promotor.

Examples of suitable terminators that are functionally linked to the coding region of the hygromycin gene are the AOX1-, nos-, PGK-, TrpC- or the CYC1-terminator (Degryse et al., Yeast 1995 Jun 15; 11(7):629-40; Brunelli et al. Yeast 1993 Dec9(12): 1309-18; Frisch et al., Plant Mol. Biol. 27 (2), 405-409 (1995); Scorer et al., Biotechnology (N.Y.) 12 (2), 181-184 (1994), Genbank acc. number Z46232; Punt et al., (1987) Gene 56 (1),

117-124)), preferably the CYC1- or nos-terminator, more preferably the nos-terminator.

In a preferred embodiment, the hygromycin cassette comprises a GPD-1 promotor functionally linked to the coding region of the hygromycin region which is functionally linked to the nos-terminator.

A functional linkage is understood to mean the sequential arrangement of promoter and coding sequence, of coding sequence and terminator or of promoter, coding sequence and terminator in such a manner that each of the regulatory elements can, upon expression of the coding sequence, fulfil its function for the recombinant expression of the nucleic acid sequence. Direct linkage in the chemical sense is not necessarily required for this purpose. Preferred arrangements are those in which the hygromycin gene to be expressed recombinantly is positioned downstream of the sequence which acts as promoter, so that the two sequences are linked covalently to each other. The distance between the promoter sequence and the nucleic acid sequence to be expressed recombinantly is preferably less than 100 base pairs, especially preferably less than 50 base pairs, and very especially preferably less than 10 base pairs. The distance between the terminator sequence and the nucleic acid sequence to be expressed recombinantly is preferably less than 100 base pairs, especially preferably less than 50 base pairs, and very especially preferably less than 10 base pairs. However, further sequences which, for example, exert the function of a linker with certain restriction enzyme cleavage sites, or of a signal peptide, may also be positioned between the two sequences.

These vectors are not only much more smaller than the currently used plasmid vectors, but also exhibit a high transformation efficiency. Surprisingly, a high transformation efficiency can be gained even if small DNA-fragments of at least 300bp, preferably at least 400bp, more preferably at least 450bp, most preferably at least 500bp of the nucleic acid sequence d) to be analyzed are used. The average degree of illegitimate recombination is below 30%, preferably below 25%, more preferably 20% and most preferably between 0 and 15%.

The nucleic acid sequence d) has a homology of at least 80% to the nucleic acid sequence of the filamentous fungi to be transformed, preferably at least 90%, more preferably at least 95% and most preferably at least 100%.

In a preferred embodiment, the nucleic acid sequence d) originates from a filamentous fungi and has a length of at least 300bp, preferably 400bp, more preferably at least 450bp, and most preferably at least 500bp. These lengths are suitable for functional genomic studies for which a high number of transformants is required. Also nucleic acid sequences exceeding 500bp can be used, e.g. for the purpose of recombinant expression.

If the nucleic acid sequence d) is to be expressed recombinantly in the filamentous fungi, it can be functionally linked to a promotor e) and optionally to a terminator f).

Examples of suitable promotors e) are for example the AUG1-, GPD-1-, PX6-, TEF-, CUP1-, PGK-, GAP1-, TPI, PHO5-, AOX1, GAL10/CYC-1, CYC1, OliC-, ADH-, TDH-, Kex2-, MFα- or the NMT-promotor or combinations of the aforementioned promotors (Degryse et al., Yeast 1995 Jun 15; 11(7):629-40; Romanos et al. Yeast 1992 Jun;8(6):423-88; Benito et al. Eur. J. Plant Pathol. 104, 207-220 (1998); Cregg et al. Biotechnology (N Y) 1993 Aug;11(8):905-10; Luo X., Gene 1995 Sep 22;163(1):127-31; Nacken et al., Gene 1996 Oct 10;175(1-2): 253-60; Turgeon et al., Mol Cell Biol 1987 Sep;7(9):3297-305).

Examples of suitable terminators f) are the NMT-, Gcy1-, TrpC-, AOX1-, nos-, the PGK- or the CYC1-terminator (Degryse et al., Yeast 1995 Jun 15; 11(7):629-40; Brunelli et al. Yeast 1993 Dec9(12): 1309-18; Frisch et al., Plant Mol. Biol. 27 (2), 405-409 (1995); Scorer et al., Biotechnology (N.Y.) 12 (2), 181-184 (1994), Genbank acc. number Z46232; Zhao et al. Genbank acc number : AF049064; Punt et al., (1987) Gene 56 (1), 117-124).

The nucleic acid sequence d) can be also functionally linked to an affinity tag to purify the encoded protein and/or to a reporter gene to study biochemical properties of the nucleic acid sequence d) in vivo, respectively.

"Reporter genes" encode readily quantifiable proteins. Using these genes, an assessment of transformation efficacy or of the site or time of expression can be made via growth, fluorescence, chemoluminescence, bioluminescence or resistance assay or via photometric measurement (intrinsic color) or enzyme activity. Very especially preferred in this context are reporter proteins (Schenborn E, Groskreutz D. Mol. Biotechnol. 1999; 13(1):29-44) such as the "green fluorescence protein" (GFP) (Gerdes HH and Kaether C, FEBS Lett. 1996; 389(1):44-47; Chui WL et al., Curr. Biol. 1996, 6:325-330; Leffel SM et al., Biotechniques.

23(5):912-8, 1997), chloramphenicol acetyl transferase, a luciferase (Giacomin, Plant Sci. 1996, 116:59-72; Scikantha, J. Bact. 1996, 178:121; Millar et al., Plant Mol. Biol. Rep. 1992 10:324-414), and luciferase genes, in general β-galactosidase or β-glucuronidase (Jefferson et al., EMBO J. 1987, 6, 3901-3907), the Ura3 gene, the Ilv2 gene, the 2-desoxyglucose-6-phosphate phosphatase gene, β-lactamase gene, the neomycin phosphotransferase gene, the hygromycin phosphotransferase gene, or the BASTA (= gluphosinate) resistance gene.

The term "affinity tag" denotes a peptide or polypeptide whose coding nucleic acid sequence can be fused to the nucleic acid sequence d) either directly or using a linker, by classical cloning techniques. The affinity tag serves to isolate the recombinant target protein by means of affinity chromatography. The abovementioned linker can optionally comprise a protease cleavage site (for example for thrombin or factor Xa), whereby the affinity tag can be cleaved off from the target protein, as required. Examples of customary affinity tags are the "his-tag", for example from Quiagen, Hilden, "strep-tag", "myc-tag" (Invitrogen, Carlsberg), New England Biolab's tag which consists of a chitin binding domain and an intein, and what is known as the CBD-tag from Novagen.

In a particularly preferred embodiment, the plasmid vector comprises an coli E1 ori, the ampicillin resistance gene as selection marker, a GPD-1 promotor functionally linked to the coding region of the hygromycin resistance gene which is functionally linked to the nos-terminator.

Preferably, the vector also comprises a multiple cloning site comprising an appropriate restriction enzyme site. Appropriate restriction sites are well known by the skilled artisan.

In a further preferred embodiment, the plasmid vector additionally comprises a TA-cloning site to facilitate the overall cloning procedure.

Examples of particularly preferred embodiments are set forth in Fig. 1 and 2.

All of the above mentioned embodiments of plasmid vectors are hereinbelow termed "plasmid vector (or vector) according to the invention".

A vector according to the invention may also comprise at least one additional selection marker.

If a plasmid is used for recombinant expression in host organisms, a marker is required indicating the sucessful transfer of the plasmid vector DNA into the filamentous fungi to be transformed.

Surprisingly, we have found that the gene fragments of the polyketide synthase are a my suitable selection marker. The term "selection marker" referring to the polyketide synthase herein means a nucleic acid sequence.

More precisely, the term "selectable marker", "selection marker" or "marker" used in connection with polyketide synthetase for transformation of filamentous fungi means a nucleic acid sequence encoding a polyketide synthetase or fragments of the aforementioned nucleic acid sequence. Preferred embodiments of the aforementioned marker as well as preferred embodiments of methods of use of the respective marker are described herein below.

Polyketide synthases are multifunctional enzymes that are involved in the biosynthesis of several important polyketides. Polyketides constitute a large and highly diverse group of secondary metabolites, synthesized by bacteria, fungi and plants and algae. They include antibiotics, compounds with mycotoxic activity, and compounds within pigment biosynthetic pathways. Furthermore a polyketide synthase is described to be required for fungal virulence of Cochliobolus heterostrophous toward maize (Yang et al., 1996 PMID:8953776). Polyketide Synthetases are furthermore known from Wangiella dermatidis (pubMedID:11179356), from Aspergillus nidulans (Swiss-prot ID: Q03149) and from Aspergillus parasiticus (Swiss-Prot ID:Q12053).

The use of polyketide synthase as selectable marker for recombinant expression in filamentous fungi has not yet been described.

The present invention also encompasses a selection marker comprising a nucleic acid sequence encoding a polyketide synthetase fragment, wherein said nucleic acid sequence comprises

i. a nucleic acid sequence shown in SEQ ID NO:1 or SEQ ID NO:2; or

ii. parts of the nucleic acid sequence as defined in i. consisting of at least 300bp.

Parts or segments of nucleic acid sequences set forth in ii. consist of at least 300bp, preferably at least 400bp, more preferably at least 450bp, most preferably at least 500bp of the nucleic acid sequences. In a further preferred embodiment, those parts are selected from SEQ ID NO:1, preferably from 732bp to 5881bp of SEQ ID NO:1 e.g. from 2236bp to 2870bp.

Furthermore, the present invention encompasses a selection marker comprising a nucleic acid sequence encoding a polyketide synthetase or a polyketide synthetase fragment, wherein said nucleic acid sequence comprises

i. a nucleic acid sequence shown in SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5; or

ii. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:6 by back translation; or

iii. a functional equivalent of the nucleic acid sequence set forth in i) which is encoded by an amino acid sequence that has at least an identity of 50% with the SEQ ID NO:6; or

iv. parts of the nucleic acid sequence as defined in i., ii. or iii. consisting of at least 300bp.

v. parts of the nucleic acid sequence as defined in i., ii. or iii. consisting of at least 300bp comprising

a) a nucleic acid sequence shown in SEQ ID NO:7; or

b) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:8 by back translation; or

c) a functional equivalent of a nucleic acid sequence set forth in a) which is encoded by an amino acid sequence that has at least an identity of 85% with the SEQ ID NO:8.

Parts or segments of nucleic acid sequences set forth in iii. or v. consist of at least 300bp, preferably at least 400bp, more preferably at least 450bp, most preferably at least 500bp of the nucleic acid sequences. Preferably, the aforementioned parts or segments of nucleic acid sequences are those set forth in v.a), v.b) or v.c), more preferably those set forth in v.a) or v.b) and most preferably those set forth in v.a). For example, those parts

can be selected from 2234bp to 2865bp of SEQ ID NO:3.

The functional equivalents of the nucleic acid sequence set forth in iv. are encoded by an amino acid sequence that has at least an identity of 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64% or 65% or preferably of 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78% or 79% more preferably of 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90% most preferably of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% with SEQ ID NO:6.

The functional equivalents of the nucleic acid sequence set forth in v.c) are encoded by an amino acid sequence that has at least an identity of 85%, 86%, 87% or 88% or preferably of 89%, 90%, 91%, 92% or 93% more preferably of 94%, 95% or 96% most preferably of 97%, 98% or 99% with SEQ ID NO:8.

Preferred are nucleic acid sequences as defined above originating from filamentous fungi, preferably phytopathogenic filamentous fungi selected from the group consisting of the genera Neurospora, Alternaria, Podosphaera, Sclerotinia, Physalospora, Botrytis, Corynespora; Colletotrichum; Diplocarpon; Elsinoe; Diaporthe; Sphaerotheca; Cinula, Cercospora; Erysiphe; Sphaerotheca; Leveillula; Mycosphaerella; Phyllactinia; Gloesporium; Gymnosporangium, Leptotthrydium, Podosphaera; Gloedes; Cladosporium; Phomopsis; Phytopora; Phytophthora; Erysiphe; Fusarium; Verticillium; Glomerella; Drechslera; Bipolaris; Personospora; Phaeoisariopsis; Spaceloma; Pseudocercosporella; Pseudoperonospora; Puccinia; Typhula; Pyricularia; Rhizoctonia; Stachosporium; Uncinula; Ustilago; Gaeumannomyces and Fusarium, more preferred from the group consisting of the genera and species Alternaria, Podosphaera, Sclerotinia, Physalospora such as Physalospora canker, Botrytis species such as Botrytis cinerea, Corynespora such as Corynespora melonis; Colletotrichum; Diplocarpon such as Diplocarpon rosae; Elsinoe such as Elsinoe fawcetti, Diaporthe such as Diaporthe citri; Sphaerotheca; Cinula such as Cinula neccata, Cercospora; Erysiphe such as Erysiphe cichoracearum and Erysiphe graminis; Sphaerotheca such as Sphaerotheca fuliginea; Leveillula such as Leveillula taurica; Mycosphaerella; Phyllactinia such as Phyllactinia kakicola; Gloesporium such as Gloesporium kaki; Gymnosporangium such as Gymnosporangium yamadae, Leptotthrydium such as Leptotthrydium pomi, Podosphaera such as Podosphaera leucotricha; Gloedes such as Gloedes pomigena; Cladosporium such as Cladosporium carpophilum; Phomopsis; Phytopora; Phytophthora such as Phytophthora infestans; Verticillium; Glomerella such as Glomerella cingulata; Drechslera; Bipolaris; Personospora; Phaeoisariopsis such as Phaeoisariopsis vitis; Spaceloma such as Space-

loma ampelina; Pseudocercosporella such as Pseudocercosporella herpotrichoides; Pseudoperonospora; Puccinia; Typhula; Pyricularia such as Pyricularia oryzae; Rhizoctonia; Stachosporium such as Stachosporium nodorum; Uncinula such as Uncinula necator; Ustilago; Gaeumannomyces species such as Gaeumannomyces graminis and Fusarium such as Fusarium dimerium, Fusarium merismoides, Fusarium lateritium, Fusarium decemcellulare, Fusarium poae, Fusarium tricinctum, Fusarium sporotrichioides, Fusarium chlamydosporum, Fusarium moniliforme, Fusarium proliferatum, Fusarium anthophilum, Fusarium subglutinans, Fusarium nygamai, Fusarium oxysporum, Fusarium solani, Fusarium culmorum, Fusarium sambucinum, Fusarium crookwellense, Fusarium avenaceum ssp. avenaceum, Fusarium avenaceum ssp. aywerte, Fusarium avenaceum ssp. nurragi, Fusarium hetrosporum, Fusarium acuminatum ssp. acuminatum, Fusarium acuminatum ssp. armeniacum, Fusarium longipes, Fusarium compactum, Fusarium equiseti, Fusarium scripi, Fusarium polyphialidicum, Fusarium semitectum and Fusarium beomiforme and especially preferred from the genera Fusarium such as Fusarium graminearum, most preferred from the group consisting of the genera and species Fusarium, Fusarium dimerium, Fusarium merismoides, Fusarium lateritium, Fusarium decemcellulare, Fusarium poae, Fusarium tricinctum, Fusarium sporotrichioides, Fusarium chlamydosporum, Fusarium moniliforme, Fusarium proliferatum, Fusarium anthophilum, Fusarium subglutinans, Fusarium nygamai, Fusarium oxysporum, Fusarium solani, Fusarium culmorum, Fusarium sambucinum, Fusarium crookwellense, Fusarium avenaceum ssp. avenaceum, Fusarium avenaceum ssp. aywerte, Fusarium avenaceum ssp. nurragi, Fusarium hetrosporum, Fusarium acuminatum ssp. acuminatum, Fusarium acuminatum ssp. armeniacum, Fusarium longipes, Fusarium compactum, Fusarium equiseti, Fusarium scripi, Fusarium polyphialidicum, Fusarium semitectum and Fusarium beomiforme, wherein Fusarium graminearum is most preferred.

Preferred non-phytopathogenic filamentous fungi are fungi of the group consisting of the genera Neurospora such as Neurospora crassa, Aspergillus such as Aspergillus parasiticus, Aspergillus nidulans, Aspergillus niger and Wangiella such as Wangiella dermatidis.

The term "comprising" means that the nucleic acid sequence according to the invention can be flanked by additional nucleic acid sequences that have on the 5' end a sequence length of at least 1000 bp and preferably at least 500 bp, more preferably at least 100bp, most preferably at least 50bp and on the 3' a sequence length of at least 1000 bp and preferably at least 500 bp,

more preferably at least 100 bp and most preferably at least 50bp.

"Functional equivalents" in the present context describe nucleic acid sequences which hybridize under standard conditions with the nucleic acid sequence or portions of the nucleic acid sequence having the function of the a selection marker.

It is advantageous to use short oligonucleotides of a length of 10-50 bp, preferably 15-40 bp, for example of the conserved or other regions, which can be determined via comparisons with other related genes in a manner known to the skilled worker for the hybridization. Alternatively, it is also possible to use longer fragments of the nucleic acids according to the invention or the complete sequences for the hybridization. These standard conditions vary depending on the nucleic acid used, viz. oligonucleotide, longer fragment or complete sequence, or depending on which type of nucleic acid, viz. DNA or RNA, is being used for the hybridization. Thus, for example, the melting temperatures for DNA:DNA hybrids are approx. 10°C lower than those of DNA:RNA hybrids of equal length.

Standard conditions are understood to mean, depending on the nucleic acid, for example temperatures between 42 and 58°C in an aqueous buffer solution with a concentration of between 0.1 and 5 x SSC (1 x SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7.2) or additionally in the presence of 50% formamide such as, for example, 42°C in 5 x SSC, 50% formamide. The hybridization conditions for DNA:DNA hybrids are advantageously 0.1 x SSC and temperatures of between approximately 20°C and 45°C, preferably between approximately 30°C and 45°C. The hybridization conditions for DNA:RNA hybrids are advantageously 0.1 x SSC and temperatures of between approximately 30°C and 55°C, preferably between approximately 45°C and 55°C. These temperatures stated for the hybridization are melting temperature values which have been calculated by way of example for a nucleic acid with a length of approx. 100 nucleotides and a G + C content of 50% in the absence of formamide. The experimental conditions for DNA hybridization are described in specialist textbooks of genetics such as, for example, Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989 and can be calculated using formulae known to the skilled worker, for example as a function of the length of the nucleic acids, the type of the hybrids or the G + C content. The skilled worker can find more information on hybridization in the following textbooks: Ausubel et al. (eds), 1985, Current Protocols in Molecular Biology, John Wiley & Sons, New York; Hames and Higgins (eds),

1985, Nucleic Acids Hybridization: A Practical Approach, IRL Press at Oxford University Press, Oxford; Brown (ed), 1991, Essential Molecular Biology: A Practical Approach, IRL Press at Oxford University Press, Oxford.

A functional equivalent is furthermore also understood to mean, in particular, natural or artificial mutations of the relevant nucleic acid sequences of the polyketide synthetase (PKS) as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5 and its homologs from other organisms , wherein mutations comprise substitutions, additions, deletions, inversions or insertions of one or more nucleotide residues. This may also lead to a modification of the corresponding amino acid sequence of the PKS by substitution, insertion or deletion of one or more amino acids.

Thus, the scope of the present invention also extends to, for example, those nucleotide sequences which are obtained by modification of the nucleic acid sequence of the selection marker described by SEQ ID NO:1 or by SEQ ID NO:2 or SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 respectively. The purpose of such a modification can be, for example, the insertion of further cleavage sites for restriction enzymes, the removal of excess DNA, or the addition of further sequences. Said nucleic acid sequences should still maintain the desired function as marker for targeted transformation, despite the deviating nucleic acid sequence.

The term "identity" or "homology" between two nucleic acid sequences or polypeptide sequences is defined by the identity of the nucleic acid sequence/polypeptide sequence by in each case the entire sequence length, which is calculated by alignment with the aid of the program algorithm GAP (Wisconsin Package Version 10.0, University of Wisconsin, Genetics Computer Group (GCG), Madison, USA), setting the following parameters:

Gap Weight: 8 Length Weight: 4

Average Match: 2,912 Average Mismatch:-2,003

The term homology when used herein is the same as the term identity.

Functional equivalents thus encompass naturally occurring variants of the sequences described herein, and also artificial, for example chemically synthesized, nucleic acid sequences adapted to

the codon usage, or the amino acid sequences derived therefrom.

Moreover, SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5, nucleic acid sequences derived from the amino acid sequence SEQ ID NO:6 by back translation or parts of the aforementioned nucleic acid sequences can be used for the detection and isolation of functional equivalents of other fungi on the basis of sequence identities. In this context, part or all of the sequence of the SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5 or nucleic acid sequences derived from the amino acid sequence SEQ ID NO:6 by back translation can be used as probe (e.g. hybridization probe) for screening in a genomic library or a cDNA library of the fungal species in question or in a computer search for sequences of functional equivalents in electronic databases. Especially for computer search for sequences of functional equivalents in electronic databases, the amino acid sequence SEQ ID NO:6 or parts of the amino acid sequence SEQ ID NO:6 are useful.

For the preparation of hybridization probes, SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5 or parts of the aforementioned nucleic acid sequences can be used. The preparation of these probes and the experimental procedure are known. For example, this can be effected via the tailor-made preparation of radioactive or nonradioactive probes by means of PCR and the use of suitably labeled oligonucleotides, followed by hybridization experiments. The technologies required for this purpose are given, for example, in T. Maniatis, E.F. Fritsch and J. Sambrook, "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989). The probes in question can furthermore be modified by standard technology (lit. SDM or random mutagenesis) in such a way that they can be employed for other purposes, for example as probe which hybridizes specifically with mRNA and the corresponding coding sequences in order to analyze the corresponding sequences in other organisms.

Furthermore, the cDNA could be used to engineer recombinant microorganisms to produce polyketide agents of pharmaceutical or agricultural interest as described by Pfeifer et al. (Pfeifer BA, Admiraal SJ, Gramajo H, Cane DE, Khosla C., Science 2001 Mar 2;291(5509):1790-2). Thus, the present invention also comprises polypeptides with the biological acitivity of a polyketide synthetase encoded by a nucleic acid sequence comprising

i. a nucleic acid sequence shown in SEQ ID NO:5 or

ii. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:6 by back translation; or

iii. nucleic acid sequence which is encoded by a functional analogue of an amino acid sequence that has at least an identity of 50% with the SEQ ID NO:6.

The term "functional analogues" describes nucleic acid sequences which are capable of bringing about the expression, in a filamentous fungi, of a polypeptide with the biological activity of polyketide synthetase and which can be deduced from an amino acid sequence by back translation which has a defiened degree of identity with SEQ ID NO:6. The functional analogues set forth in iii) have at least an identity of 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64% or 65% or preferably of 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78% or 79% more preferably of 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90% most preferably of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% with the SEQ ID NO:6.

Thus, the present invention also encompasses, for example, those nucleotide sequences which are obtained by modification of the abovementioned nucleic acid sequences. For example, such modifications can be generated by techniques with which the skilled worker is familiar, such as "site directed mutagenesis", "error prone PCR", "DNA shuffling" (Nature 370, 1994, pp.389-391) or "staggered extension process" (Nature Biotechnol. 16, 1998, pp.258-261). The purpose of such a modification can be, for example, the insertion of further cleavage sites for restriction enzymes, the removal of DNA in order to truncate the sequence, the substitution of nucleotides in order to optimize the codons, or the addition of further sequences. Proteins which are encoded via modified nucleic acid sequences must retain the desired functions despite a deviating nucleic acid sequence.

Functional analogues thus comprise naturally occuring variants of the herein-described sequences and artificial nucleic acid sequences, for example those which have been obtained by chemical synthesis and which are adapted to the codon usage, and also the amino acid sequences derived from them.

As explained above, also the expression cassette or the vector comprising a PKS encoding nucleic acid sequence may comprise at least an additional selection marker, preferably the hygromycin resistance gene so that in a particular preferred embodiment, the selection of the succesfully transformed filamentous fungi can be

carried out by hygromycin resistance of successfully transformed clones and by the presence of pigment (color) of successfully transformed clones. Most preferably, the vector comprising the PKS encoding nucleic acid sequence is a vector according to the invention comprising a PKS encoding nucleic acid sequence. In addition to the aforementioned selection method homologous recombination can be confirmed by PCR based on oligonucleotides preferably derived from the vector sequence flanking the 5 and 3 region of the gene to be inserted. Specific examples of these primers are given in the examples.

The invention furthermore relates to the use of polyketide synthetase encoding nucleic acid sequences as marker for targeted transformation in filamentous fungi.

Preferably, the present invention comprises the use of a nucleic acid sequence comprising

a) a nucleic acid sequence encoding a polyketide synthetase; or

b) parts of the nucleic acid sequence as defined in i., ii. or iii. consisting of at least 300bp

for transformation of filamentous fungi.

Preferred is the use of a nucleic acid sequence as marker for targeted transformation in filamentous fungi said nucleic acid comprising

i. a nucleic acid sequence shown in SEQ ID NO:1 or SEQ ID NO:2; or

ii. parts of the nucleic acid sequence as defined in i. consisting of at least 300bp, preferably at least 400bp, more preferably at least 450bp and most preferably at least 500bp;

Equally preferred is the use of a nucleic acid sequence as marker for targeted transformation in filamentous fungi, said nucleic acid comprising

iii. a nucleic acid sequence shown in SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5; or

iv. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:6 by back translation; or

v. a functional equivalent of the nucleic acid sequence set forth in i) or iii) which is encoded by an amino acid sequence that has at least an identity of 40% with the SEQ ID NO:6; or

vi. a nucleic acid sequence shown in SEQ ID NO:9 or SEQ ID NO: 11;

vii. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:13 by back translation; or

viii. parts of the nucleic acid sequence as defined in iii., iv, v., vi or vii. consisting of at least 300bp, preferably at least 400bp, more preferably at least 450bp and most preferably at least 500bp; or

ix. parts of the nucleic acid sequence as defined in iii., iv, v., vi or vii. consisting of at least 300bp, preferably at least 400bp, more preferably at least 450bp, and most preferably at least 500bp comprising

a) a nucleic acid sequence shown in SEQ ID NO:7; or

b) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:8 by back translation; or

c) a functional equivalent of a nucleic acid sequence set forth in a) which is encoded by an amino acid sequence that has at least an identity of 68% with the SEQ ID NO:8.

The nucleic acid sequences according to i. to ix encode for a polypeptide with the biological function of a polyketide synthetase or for a fragment of the aforementioned polypeptide.

Under the aforementioned sequences, the nucleic acid sequences according to i., ii., iii., iv., v. as well as parts of the aforementioned nucleic acid sequence consisting of at least 300bp, preferably at least 400bp, more preferably at least 450bp, most preferably at least 500bp are preferred. Those parts are preferably those set forth in ix.

Preferred phytopathogenic and non-phytopathogenic filamentous fungi are those mentioned above. The aforementioned nucleic acid sequences are hereinbelow also termed "PKS marker". Preferably, the term "PKS marker" designates nucleic acid sequences according to i., ii., iii., iv., v. as well as parts of the aforementioned nucleic acid sequence consisting of at least 300bp, preferably at least 400bp, more preferably at least 450bp, and most preferably at least 500bp are preferred.

The functional equivalents of the nucleic acid sequence set forth in iv. can be deduced from a functional equivalent of the amino acid sequence shown in SEQ ID NO:6 by back translation having at least an identity of 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48% or 49% preferably of 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78% or 79% more preferably of 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90% and most preferably of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% with the SEQ ID NO:6.

The functional equivalents of the nucleic acid sequence set forth in ix.c) can be deduced from a functional equivalent of the amino acid sequence shown in SEQ ID NO:8 by back translation having at least an identity of 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77% or 78% preferably of 79%, 80%, 81%, 82%, 83%, 84% or 85% more preferably of 86%, 87%, 88%, 89% or 90% most preferably of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% with the SEQ ID NO:8.

The use of a PKS marker for targeted transformation of filamentous fungi can be based on significant reduction in the amount of polyketide synthetase which is present in a filamentous fungi. A reduction in the amount the polyketide synthetase means that the amount of polypeptide is reduced via recombinant methods.

Reduction via recombinant methods can involve "antisense techniques", which describes a technology for the suppression (reduction) of expression of polyketide synthetase, where a PKS marker is transformed into the respective filamentous fungi in "antisense" orientation under the control of a suitable promoter. This method is used preferably for Aspergillus species, and more preferrably for Aspergillus nidulans. The technologies required herefore are well known by the skilled artisan (for example see Bautista et al., Appl. Environ. Microbiol. 2000; 66(10) 4579-81). Suitable vectors therefore comprise an expression cassette comprising

a) a promotor sequence in functional linkage with a PKS marker in antisense orientation; and optionally

b) further genetic control sequences functionally linked to a nucleic acid sequence according to a).

The afore-mentioned expression cassette is hereinbelow termed as "PKS Marker expression cassette".

The term "expression cassette" can be defined as follows: An expression cassette comprises a nucleic acid sequence which should be expressed, linked functionally to at least one genetic control element, such as a promoter, and, advantageously, a further control element, such as a terminator. Examples of suitable promotors and terminators are given above. The nucleic acid sequence of the expression casette can be, for example, a genomic or complementary DNA sequence or an RNA sequence, and the semisynthetic or fully synthetic analogs thereof. These sequences can exist in linear or circular form, extrachromosomally or integrated into the genome. The nucleic acid sequences in question can be synthesized or obtained naturally or comprise a mixture of synthetic and natural DNA components, and consist of a variety of heterologous gene segments from various organisms.

Artificial nucleic acid sequences are also suitable in this context as long as they make possible the expression, in a cell or organism, of a polypeptide encoded by a nucleic acid sequence according to the invention and having the biological activity of a polyketide synthetase. For example, synthetic nucleotide sequences can be generated which have been optimized with regard to the codon usage of the organisms to be transformed.

All of the abovementioned nucleotide sequences can be generated from the nucleotide units by chemical synthesis in the manner known per se, for example by fragment condensation of individual, overlapping complementary nucleotide units of the double helix. Oligonucleotides can be synthesized chemically for example in the manner known per se using the phosphoamidite method (Voet, Voet, 2nd Edition, Wiley Press New York, pp. 896-897). When preparing an expression cassette, various DNA fragments can be manipulated in such a way that a nucleotide sequence with the correct direction of reading and the correct reading frame is obtained. The nucleic acid fragments are linked to each other via general cloning techniques as are described, for example, in T. Maniatis, E.F. Fritsch and J. Sambrook, "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989) and in T.J. Silhavy, M.L. Berman and L.W. Enquist, Experi-

ments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984) and in Ausubel, F.M. et al., "Current Protocols in Molecular Biology", Greene Publishing Assoc. and Wiley-Interscience (1994).

The term "genetic control element" describes sequences which have an effect on the transcription and, if appropriate, translation of the nucleic acids according to the invention in prokaryotic or eukaryotic organisms. Examples are terminators. Examples of suitable terminators are given above. In addition to the afore-mentioned control sequences, or instead of these sequences, the natural regulation of these sequences may still be present before the actual structural genes and may, if appropriate, have been modified genetically in such a way that the natural regulation has been switched off and the expression of the target gene has been modified, that is to say increased or reduced. The choice of the control sequence depends on the host organism or starting organism. Genetic control sequences furthermore also comprise the 5'-untranslated region, introns or the noncoding 3' region of genes. Control sequences are furthermore understood as meaning those which make possible a homologous recombination or insertion into the genome of a host organism or which permit the removal from the genome. Genetic control sequences also comprise further promoters, promoter elements or minimal promoters.

The transcription of the PKS marker leads to suppression of the transcription of the natural polyketide synnthethase gene, which can be detected by loss of color of the transformed fungi relative to the respective wild-type strain.

In a preferred embodiment, the reduction via recombinant methods is based on a gene knock out of the polyketide synthethase gene using either an expression cassette additionally comprising the PKS marker or a vector comprising the PKS marker in the respective filamentous fungi. Disruption of the PKS marker will lead to a loss of color.

Preferred phytopathogenic filamentous fungi are those mentioned above. Preferred non-phytopathogenic filamentous fungi are fungi of the group consisting of the genera Aspergillus such as Aspergillus parasiticus, Aspergillus nidulans and Wangiella such as Wangiella dermatidis.

In this connection, the selection of the functional equivalent for the use as marker gene depends on the fungi to be transformed. By preference, the polyketide synthetase fragment has an identity of at least 80%, preferably at least 81%, 82%, 83%, 84%,

85%, 86%, 87%, 88%, 89%, 90%, and especially preferably at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity with the polyketide synthetase of the fungi to be transformed.

For example, for transformation of Fusarium graminearum, a nucleic acid sequence can be selected comprising

i. a nucleic acid sequence shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5; or

ii. a nucleic acid sequence that has at least an identity of 80% SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5; or

iii. parts of the nucleic acid sequence as defined in i. or ii. consisting of at least 300bp, preferably at least 400bp, more preferably at least 450bp, and most preferably at least 500bp.

iv. parts of the nucleic acid sequence as defined in i. or ii. consisting of at least 300bp, preferably at least 400bp, more preferably at least 450bp, and most preferably at least 500bp comprising

a) a nucleic acid sequence shown in SEQ ID NO:7; or

c) a nucleic acid sequence that has at least an identity of 80% with the SEQ ID NO:8.

As mentioned above, another embodiment of the present invention is plasmid vectors for targeted transformation of filamentous fungi comprising a PKS marker. These plasmid vectors are either vectors currently used for targeted transformation of filamentous fungi e.g. such as pAN7 (Punt et al, 1987 Gene 36:117-124) and other vectors that are well known by the skilled artisan or plasmid vectors according to the invention, preferably plasmid vectors according to the invention.

All of the above-mentioned vectors comprising the PKS marker are hereinbelow termed as "PKS vectors".

A PKS vector is also a vector, which comprises a PKS Marker-expression cassette.

All vectors according to the invention not comprising the PKS marker are hereinbelow termed as "non-PKS vectors".

The present invention furthermore encompasses a method for preparing mutated filamentous fungi, comprising the steps of transferring a non-PKS vector or a PKS vector into a filamentous fungi; and selecting clones of said filamentous fungi, which contain at least one genetic marker introduced by said plasmid vector.

The term filamentous fungi as well as preferred fungi for this purpose are those mentioned and defined above.

In a preferred embodiment, the method for preparing mutated filamentous fungi, comprising the following steps

a) transferring a PKS vector into a filamentous fungi; and

b) selecting successfully transformed filamentous fungi by the absence of color (pigment).

As explained above, the absence of color is based on significant reduction in the amount of polyketide synthetase (or in the polyketide syntetehase activity or instability of polyketide syntethase mRNA, which is present in a filamentous fungi). The absence of color can be monitored for example by comparing the transformed fungi with the respective wild-type fungi of the same species.

If a PKS vector is transferred into a filamentous fungi, the disruption of the PKS gene leads to a loss of color (pigment) whereby the degree of transformation can be determined easily. Resulting transformants are white in contrast to the colored wild-type. Thus, the selection according to step b) is done by monitoring the absence of color (pigment) in the filamentous fungi. In a preferred embodiment, the absence of pigment is monitored by optical means.

Alternatively, the absence of color results from the reduction of the polyketide synthethase via antisense techniques. The absence of color hereby means a "reduction of color" or, preferably, loss of color. Absence of color means a reduction in color of at least 20%, preferably between 20 and 40%, more preferably between 40 and 60%, especially preferaby between 60 and 80% and most preferably between 80% and 100%.

In a more preferred embodiment, the PKS vector comprises at least an additional selection marker, preferably the hygromycin resistance gene. In a particular preferred embodiment, the selection of the successfully transformed filamentous fungi comprising a PKS vector can be carried out by hygromycin resistance of success-

fully transformed clones and by the absence of pigment of successfully transformed clones. Most preferably, the PKS vector is a vector according to the invention additionally comprising a PKS marker.

In a further embodiment of the invention, the selection of the succesfully transformed filamentous fungi comprising a non-PKS vector can be carried out by hygromycin resistance of successfully transformed clones.

If a non-PKS vector is used, the vector is linearized by a restriction enzyme cutting in the nucleic acid sequence region of element d). Also nucleic acid sequences exceeding 2000 bp can be used what can be disadvantageous as mentioned above. If a PKS vector is used, the plasmid vector is transferred into a filamentous fungi with the proviso that said vector is linearized by a restriction enzyme in PKS marker nucleic acid sequence. Unlike the non-PKS vectors, the nucleic acid sequence to be expressed recombinantly can also be smaller than 400bp.

In addition to the aforementioned selection methods set forth in step a) to c), homologous recombination can be confirmed by PCR based on oligonucleotides preferably derived from the vector sequence flanking the 5′ and 3′ regions of the gene to be inserted. Specific examples of these primers are given in the examples.

The plasmid vector may be transferred into the filamentous fungi to be transformed by methods familiar to the skilled worker, preferably via protoplast preparation with driselase or driselase and glucanase as lytic enzyme.

The above-mentioned transformation methods can be also realized in a high throughput screening. Using high throughput screening, many different clones are obtained in parallel so that large numbers of successfully tranformed clones of filamentous fungi can be quickly screened.

The term filamentous fungi as well as preferred fungi for this purpose are those mentioned and defined above.

Due to the convenience of the vector, the above-mentioned KO-plasmid preparation, fungi transformation and screening of the mutants can be at least partially automated so that the whole procedure can also be realized in a high throughput screening. Using high throughput system for example for KO-plasmid preparation and DNA amplification by PCR to screen the recombinant mu-

tants, many different clones are obtained in parallel so that large numbers of transformants can be quickly screened.

Mutagenized filamentous fungi, obtainable according to a method mentioned above, are further encompassed by the present invention.

In an alternative embodiment, the method of transforming filamentous fungi based on the use of polyketide synthetase as marker for transformation comprises the following steps:

a) providing a filamentous fungi characterized by the absence of color (pigment), in which the polyketide synthetase gene is modified in such a way that the polyketide synthetase cannot be functionally expressed;

b) transforming the filamentous fungi of step a) with a "sense expression cassette" or a vector comprising the aforementioned expression cassette;

c) selecting successfully transformed filamentous fungi by the presence of pigment (color).

The nucleic acid sequence as defined in b) i to v. is herein below termed as PKS encoding sequence.

The terms "expression cassette" and "genetic control elements" are explained above.

The "sense-expression cassette" set forth in step b) of the above-mentioned method comprises

a) a promotor sequence in functional linkage with a nucleic acid sequence comprising

i. a nucleic acid sequence shown in SEQ ID NO:3, 4 or 5; or

ii. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:6 by back translation; or

iii. a functional equivalent of the nucleic acid sequence set forth in i) which is encoded by an amino acid sequence shown in SEQ ID NO:6 that has at least an identity of 40% with the SEQ ID NO:6; or

iv. a nucleic acid sequence shown in SEQ ID NO:9 or SEQ ID NO: 11;

v. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:13 by back translation;

and optionally

b) further genetic control sequences in functionally linked with a nucleic acid sequence according to a).

The expression cassette or vector comprises preferably a polyketide synthetase encoding nucleic acid sequence as set forth in b) i., ii. or iii..

Preferred phytopathogenic filamentous fungi are those mentioned above. Preferred non-phytopathogenic filamentous fungi are fungi of the group consisting of the genera Aspergillus such as Aspergillus parasiticus, Aspergillus nidulans and Wangiella such as Wangiella dermatidis.

The modification of the polyketide synthetase encoding sequence of the respective fungi can be done either by introduction of at least one mutation in the gene encoding a polyketide synthetase or disruption of the gene encoding a polyketide synthetase.

The term "disruption of the PKS marker" means that the PKS marker sequence is disrupted by introducing DNA comprising stop-codons in the PKS marker sequence e.g. by homologous recombination. The respective methods are well known by the skilled artisan.

The term "mutations" of nucleic acid sequences comprises substitutions, additions, deletions, inversions or insertions of one or more nucleotide residues, which have to bring about termination of translation of the corresponding amino acid sequence of the target protein by the substitution, insertion or deletion of one or more amino acids (e.g. a by frame-shift or introduction of stop codon or amendment of nucleic acid sequence). The respective methods are well known by the skilled artisan.

For example, the mutations are carried out in the flanking regions of exon and intron of a PKS gene. These regions can be determined easily by the skilled artisan. For example, in SEQ ID NO:3 the flanking regions between exon are at bp 1022/1023; bp 1067/1068, bp 1361/1362; bp 1067/1068; bp 1361/1362; bp

1067/1068; bp 1361/1362; bp 1416/1417; bp 2399/2400; bp 2447/2448; bp 2675/2676; bp 2738/2739; bp 5744/5745; bp 5792/5793; and/or bp 7205/7206 (Ende 6. exon bp 7205).

The term "functional analogues" is defined above describe, in the present context nucleic acid sequences which are capable of bringing about the expression, in a filamentous fungi, of a polypeptide with the biological activity of polyketide synthetase and which can be deduced from an amino acid sequence by back translation which has at least an identity of 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48% or 49%, preferably of 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78% or 79%, more preferably of 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, or 90% most preferred of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% with the SEQ ID NO:6.

As explained above, the plasmid vector may be transferred into the filamentous fungi to be transformed by methods familiar to the skilled worker, preferably via protoplast preparation with driselase or driselase and glucanase as lytic enzyme.

The above-mentioned transformation methods can also be realized in a high throughput screening. Using high throughput screening, many different clones are obtained in parallel so that large numbers of successfully tranformed clones of filamentous fungi can be quickly screened.

The invention is now illustrated by the examples which follow, but is not limited thereto.

Examples

The recombinant methods on which the exemplary embodiments which follow are based are now described briefly:

A: General methods

Cloning methods such as, for example, restriction cleavages, DNA isolation, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linking of DNA fragments, transformation of E. coli cells, bacterial cultures, sequence analysis of recombinant DNA and Southern and Western Blots were carried out as described by Sambrook et al., Cold Spring Harbor Laboratory Press (1989) and Ausubel, F.M. et al., Current Protocols in Molecular Biology,

Greene Publishing Assoc. and Wiley-Interscience (1994); ISBN 0-87969-309-6.

The bacterial strains used hereinbelow (E. coli DH5 or XL1 blue) were obtained from Life Technologies or Stratagene . The vectors were used for cloning. DSM:4527 can be used as F. Graminearum wild-type strain 8/1. Restriction maps of the vectors pUCmini-Hyg and PUCmini-Hyg TA are given in Fig 1 and 2.

B: Sequence analysis of recombinant DNA

Recombinant DNA molecules were sequenced using an ABI laser fluorescence DNA sequencer following the method of Sanger (Sanger et al., Proc. Natl. Acad. Sci. USA, 74, 5463-5467(1977)). Fragments resulting from a polymerase chain reaction were sequenced and verified in order to avoid polymerase errors in constructs to be expressed.

C: Materials used

Unless otherwise specified in the text, all of the chemicals used were obtained in analytical grade quality from Fluka (Neu-Ulm), Merck (Darmstadt), Roth (Karlsruhe), Serva (Heidelberg) and Sigma (Deisenhofen). Solutions were prepared using pure pyrogen-free water, referred to in the following text as H_2O, from a Milli-Q water system purification unit (Millipore, Eschborn). Restriction enzymes, DNA-modifying enzymes and molecular-biological kits were obtained from AGS (Heidelberg), Amersham (Brunswick), Biometra (Göttingen), Roche (Mannheim), Genomed (Bad Oeynnhausen), New England Biolabs (Schwalbach/Taunus), Novagen (Madison, Wisconsin, USA), Perkin-Elmer (Weiterstadt), Pharmacia (Freiburg), Qiagen (Hilden) and Stratagene (Heidelberg). Unless otherwise specified, they were used following the manufacturer's instructions.

All of the media and buffers used for the genetic engineering experiments were sterilized either by filter sterilization or by heating in an autoclave.

In degenerated primer sequences, the following abbreviations are used:

A or T = "W";
G or C = "s";
T or C = "Y";
A or C = "M";
A or G = "R";

Examples

Example 1 – Construction of pUCmini-Hyg and PUCmini-Hyg TA vector

A 2536 bp DNA fragment corresponding to the promoter of glycerol-3-phosphate dehydrogenase (GPD1) from *Cochliobolus heterotrophus* associated to the hygromycine B resistance gene from *Escherichia coli* was amplified by PCR with the oligonucleotides

P1 5' atgaagcttggggtttgagggccaatggaacgaaactagtgtaccacttgacc 3' (SEQ ID NO 14); and

P2 5'gacagatctggcgccattcgccattcag 3' (SEQ ID NO 15)

using pGUS5 as template (Mönke, E. and Schäfer, W., 1993, Mol. Gen. Genet. 241: 73-80). The PCR is done using standard protocols; e.g. as described in Maniatis et al., Mol. Cloning.

The resulting DNA fragment was inserted in the plasmid pFDX3809 (WO 01/38504) by the restriction site Hind III and Bgl II introduced by the oligonucleotides P1 and P2. The resulting plasmid pHygB serves as template for a further PCR, wherein the Oligonucleotides

ANK 518 5' ggaatcggtcaatacactac 3' (SEQ ID NO 16)

ANK 519 5' tgtagatctctattcctttgccctcggacgagt 3' (SEQ ID NO 17)

are used to shorten the hygromycin B resistance gene specifically. The resulting PCR fragment comprising 575 bp of the 3'end of the hygromycine gene was inserted in the plasmid pHygB via the restriction sites Nde I/ Bgl II generating the plasmid pHygB-NOS.

A Hind III / Ssp I DNA fragment of 2019 bp containing the expression cassette GPD1 promoter, the hygromycine B resistance gene and the nopaline synthase terminator was isolated from pHygB-NOS and inserted in the pUCmini plasmid (= plasmid pFDX3809, see WO 01/38509) previously treated with EcoRI and HindIII restriction enzymes to give the plasmid pUCmini-Hyg; to do so , the EcoRI ends were made compatible with Ssp I by a fill-in treatment using the Klenow fragment of DNA polymerase I. A second version of pUCmini-Hyg, called pUCmini-Hyg-TA, was obtained by the insertion of the following adaptor in the NotI/AscI restriction sites of pUCmini-Hyg:

5' GGCCGCCACGGATATCTTGGCCAAAGAATTCCTGG 3' (SEQ ID NO 18)

3' CGGTGCCTATAGAACCGGTTTCTTAAGGACCGCGC 5' (SEQ ID NO 19)

The adaptor contains 2 XcmI restriction sites so that XcmI digest of pUCmini-Hyg-TA creates T-overhangs that permits direct cloning of PCR products made with the classical Taq-polymerases.

Example 2 - Construction of the PKS comprising vector "pUCmini-Hyg-PKS"

The nucleic acid sequence encoding PKS was amplified by PCR with degenerated primers

LC1 5'-GAY CCI MGI TTY TTY AAY ATG-3' (SEQ ID NO 20)

LC2c 5'-GTI CCI GTI CCR TGC ATY TC-3' (SEQ ID NO 21)

based on the conserved amino acid sequence of the PKS gene sequences from Aspergillus nidulans, Colletotrichum lagenarium, Penicillium patulum, and Aspergillus parasiticus (Bingle et al., 1999) using genomic DNA of Fusarium graminearum as template. Thermal cycling parameters consisted of an initial denaturation at 94°C for 3 min followed by 34 cycles of 94°C for 1 min (denaturation), 55°C for 1 min (annealing), 72°C for 3 min (extension) and a final extension at 72°C for 10 min according to standard procedures. The resulting PCR product was cloned into the pGEM-T vector (Promega, Mannheim, Germany) to give the plasmid pGEM-T/PKS833 and sequenced. A 633 bp DNA fragment (2236bp to 2870bp of SEQ ID NO:1; corresponding to 2234bp to 2865bp of SEQ ID NO:3; set forth in SEQ ID NO:18) was amplified by PCR using the oligonucleotides

ANK593 5' ATAAGAATGCGGCCGCAATGGCCCTCGAAACAGC 3' (SEQ ID NO 22)

ANK594 5' AAATGGCGCGCCGCGCCCAGAATGACACC 3' (SEQ ID NO 23)

and cloned into the plasmid pUCmini-Hyg using the restriction site NotI and AscI present in the oligonucleotide sequences. The resulting plasmid pUCmini-Hyg-PKS is used for homologous recombination.

The flanking regions of the PKS DNA fragment were obtained by inverse PCR (Triglia T, Peterson MG, Kemp DJ, Nucleic Acids Res 1988 Aug 25;16(16):8186). Genomic DNA was treated with the restriction enzymes PstI, NcoI, or XhoI respectively. DNA was then self-ligated to get circular DNA molecule. The latter was used as template for the inverse PCR reaction using the primers

P1A: 5′ TGCCACCTGTAGTCTGCAATCAG 3′(SEQ ID NO 24) and

P2A:5′ TGACTAACCCTGACAACTTCGCTG 3′ (SEQ ID NO 25)

deduced from the polyketide synthetase (PKS) DNA fragment of the plasmid pGEM-T/PKS833 described above.

In a second step, the PCR product was reamplified with the nested primers

P1B:5′ CCAGGATCCGACTGCTCAG 3′ (SEQ ID NO 26) and

P2B:5′ CTACATCGAGATGCACGGCAC 3′ (SEQ ID NO 27)

(deduced from the PKS DNA fragment of the plasmid pGEM-T/PKS833), cloned into the pPCR-XL-TOPO vector (Invitrogen) and sequenced to get SEQ ID NO:1.

Identification of the genomic DNA Sequence

The remaining parts of the flanking regions were obtained by Tail-PCR (Liu YG, Whittier RF; Genomics 1995 Feb 10;25(3):674-81) using 9 arbitrary degenerated primers

FJM-tail-AD1	5′-NGT CGA SWG ANA WGA A-3′ (SEQ ID NO 28),
FJM-tail-AD2	5′-GTN CGA SWC ANA WGT T-3′ (SEQ ID NO 29),
FJM-tail-AD3	5′-WGT GNA GWA NCA NAG A-3′ (SEQ ID NO 30),
FJM-tail-AD4	5′-NTC GAS TWT SGW GTT-3 (SEQ ID NO 31)′
FJM-tail-AD6	5′-TGW GNA GWA NCA SAG A-3′ (SEQ ID NO 32),
FJM-tail-AD7	5′-AGW GNA GWA NCA WAG G-3′ (SEQ ID NO 33),
FJM-tail-AD8 and	5′-CAW CGI CNG AIA SGA A-3′ (SEQ ID NO 34)
FJM-tail-AD9	5′-TCS TIC GNA CIT WGG A-3 (SEQ ID NO 35)′

coupled to the primer

TailPKS1c 5′-TTG TTA CTG GAG AGG TAA TGA AG-3′ (SEQ ID NO 36)

specific for the 5′ PKS flanking region deduced from SEQ ID NO:1,

or coupled to the primer

TailPKS2c 5'-TGA GAC AGA TCT CGC GAG CCC TC-3'.(SEQ ID NO 37)

specific for the 3' PKS flanking region deduced from SEQ ID NO:1. After subcloning and subsequent sequencing of the PCR products SEQ ID NO:3 was obtained.

Identification of the cDNA Sequence of Polyketide Synthetase

The PKS cDNA sequence was obtained by RT-PCR with a crude RNA preparation from Fusarium graminearum and various primers deduced from the genomic sequence. This was done according the classicals methods (Ausubel, F.M. et al., Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley-Interscience (1994); ISBN 0-87969-309-6). Alignement of cDNA and genomic PKS sequences permits to be identified precisely the location of introns in the genomic sequence.

Example 3 Transformation of F. graminearum

50 ml of CM-medium (Leach et al., 1982, J. Gen. Microbiol. 128: 1719-1729) were inoculated with approximately 10^5 conidia, and incubated for 2 days at 28°C, 140 rpm. Resulting hyphae were homogenized in a Warring-Blender; 200 ml CM were inoculated with 10 ml hyphal suspension, and incubated overnight at 24°C. Mycel were trapped on a sterile filter, and washed two times with sterile water. 2 g of the hyphae were resuspended in 20 ml Driselase/Glucanase (Interspex Products, San Maneo, USA; 5% / 3% in 700 mM NaCl, pH 5.6), and digested 2½ to 3 h at 28°C, 75 rpm. Undigested hyphal were removed from the protoplast suspension by filtration through gauze and Nybold membrane (50 µm pore size). The protoplast suspensions were combined with 700 mM NaCl and again passed through the gauze and the Nybold membrane. The protoplasts were pelleted by centrifugation (1300 x g) in a swing-out rotor and washed two times with ice-cold NaCl 700 mM and centrifuged (830 x g). Then the protoplasts were resuspended in STC (0.8 M sorbitol, 50 mM Tris-HCl pH 8.0, 50 mM $CaCl_2$) and stored on ice until transformation (maximum 1 week).

For transformation, protoplasts were resuspended in 4 parts STC and 1 part SPTC (0.8 M sorbitol, 40% polyethylene glycol 4000, 50 mM Tris-HCl pH 8.0, 50 mM $CaCl_2$) at a concentration of 0.5-2 x 10^8/ml; 30 µg of the pUCmini-Hyg-PKS plasmid DNA linearized with the *Eco*47III restriction site inside the PKS fragment and 5 µl heparin (5 mg/ml in STC) were added to 100 µl of the protoplast suspension in 10 ml tubes. After mixing, samples were incubated

on ice for 30 min. 1 ml SPTC was mixed with the suspension and incubated at room temperature for 20 min. Protoplasts were mixed gently into 200 ml regeneration medium (0.1% (w/v yeast extract, 0.1% (w/v. caseinhydrolysate, 34.2% (w/v) sucrose, 1.6% (w/v) granulated agar) at 43°C and spread on 94 mm plates (20 ml per plate). The plates were incubated at 28°C. After 12-24 h, the plates were overlaid with 10 ml per plate water based selective medium (16g/l granulated agar, 100mg/l hygromycin and further incubated at 28°C until transformants were obtained, which were transferred to fresh CM-Hyg-plates (consisting of CM-media, 100 µg/ml hygromycin and 2% (w/v) agar. The transformants were isolated by single spore isolation. For generation of conidia, the transformants were cultivated on SNA plates (Nirenberg, 1981, Canadian J. Botany 59: 1599-1609) under UV-light 7-14 days at 18°C. Dilutions of conidia were plated on CM-Hyg plates, and single colonies were transferred from these plates to fresh CM-Hyg plates.

Example 4 Southernblot analysis

Genomic DNA was isolated from frozen hyphal material using the Puregene Genomic DNA Isolation Kit (Gentra Systems, Minneapolis USA) and digested for 6 h with *Nru*I restriction enzyme. The genomic DNA was separated by electrophoresis on a 1% (w/v) agarose gel and blotted onto a nylon membrane (Hybond NX; Amersham Pharmacia Biotech, Buckinghamshire, England). A digoxigenin labeled probe was generated by PCR based on specific primers PKS forward 5'-GCG CTT GAG ATG GCT AGT ATC G-3' and and PKS reverse 5'-GTG CCG TGC ATC TCG ATG TAG-3' using pGEM-T/PKS833 as template and digoxigenin labeled dUTPs by PCR reaction according to the recommendation of the manufacturer (Roche Diagnostics GmbH, Mannheim). PCR conditions were 94°C for 3 min (initial denaturation) followed by 30 cycles of 94°C for 30 sec (denaturation), 55°C for 45 sec (annealing), 72°C for 1 min (extension) and a final extension at 72°C for 10 min. The non-radioactive hybridization and the detection were done under highly stringent conditions as described in Roche Molecular Biochemicals DIG Application Manual for Filter Hybridization (Roche Diagnostics GmbH, Mannheim).

To confirm the insertion of the vector construct into the PKS locus in comparison with the wild type gene, primers

EF-PKS 5' atgtctccaaaggaagctgagc 3' (SEQ ID NO 38); and

ER-PKS 5'tcgagtgatggatactgcttcg 3' (SEQ ID NO 39)

are constructed based on the PKS DNA sequence from the plasmid pGEM-T/PKS833; four universal primers are constructed, wherein

Lac 92 5' cggctacactagaaggacagtatttggta 3' (SEQ ID NO 40)

Lac 93 5' gtcaggcaactatggatgaacgaaatagac 3' (SEQ ID NO 41)

Lac 94 5' acccatctcataaataacgtcatgc 3' (SEQ ID NO 42); and

Lac 95 5' caactctatcagagcttggttga 3' (SEQ ID NO 43)

permit amplification of a 412 bp DNA fragment of the hygromycin cassette.

PCR reactions were conducted in classical conditions: 94°C for 3 min (initial denaturation) followed by 30 cycles of 94°C 60 sec (denaturation), 55°C for 90 sec (annealing), 72°C for 90 sec (extension) and a final extension at 72°C for 10 min.
Six (6) recombinant clones resistant to hygromycine were analyzed by PCR using the primer set Lac 94 / Lac 95 specific for the hygromycin resistance gene. All the mutants were found to present the expected DNA fragment of 412 bp, indicating the integration of the plasmid pUCmini-Hyg-PKS in the genome.

A 712 bp corresponding to the PKS gene could be amplified with the primer set EF-PKS/ER-PKS mentioned above using genomic DNA from a wild type strain; however no PCR fragments were amplified with genomic DNA from the recombinant clones indicating that the PKS gene is disrupted by the insertion of pUCmini-Hyg-PKS. This was confirmed by PCR amplification EF-PKS combined with Lac 93 (hybridizing to the plasmid backbone near Not I restriction site) and ER-PKS combined with Lac 92 (hybridizing to the plasmid backbone near Asc I restriction site). In both cases, DNA fragments of about 600 bp were amplified for the recombinant clones but not for the wild type strain (WT). Alltogether, the PCR analysis using the different primer sets proves that the plasmid pUCmini-Hyg-PKS was targeted specifically in the PKS locus by homologous recombination. This process permits the PKS gene to be disrupted since the recombinant mutants were found to lack the typical pigmentation (purple) of the wildtype strain.

Example 5 functional expression of Green Fluorescent Protein (GFP) in Fusarium graminearum

A) Plasmid construction

In a first step, a 67bp DNA fragment encoding the peptide leader of the first 23 amino acids from N-terminus of the yeast ARH1 (SwissProt;P48360) was amplified by PCR using the primers

Lac 80 5′ cccgaattcatgagctttgttcaaataagg 3′ (SEQ ID NO 44) and

Lac 81 5′ ttattctagattttccatgggaatggatacagtcttacg 3′ (SEQ ID NO 45)

In a second step, a 734 bp DNA fragment encoding the Green Fluorescent Protein (GFP) was amplified by PCR using the plasmid pEGFP-N2 (Genbank; U57608) and the primers

Lac 84 5′ cgccaccatggtgagcaagggcgaggagctgtt 3′ (SEQ ID NO 46) and

Lac 85 5′ tatgatctagagtcgcggccgctttacttgtacagctcg 3′ (SEQ ID NO 47).

The PCR products were assembled in frame with the Nco I restriction sites present in the oligonucleotides Lac 81 and Lac 84 and cloned in the expression plasmid pYes2 (Invitrogen) using the restriction sites EcoRI and Xba I present in the oligonucleotides Lac 80 and Lac 85, respectively. In the resulting plasmid pLAC7, the recombinant gene encoding GFP is under the control of the galactose (Gal 1) promoter and cytochrome C1 terminator.

A 2892 bp DNA fragment containing the GFP expression cassette was isolated from pLac7 using the restriction sites Nae I and Bsa I and cloned in the plasmid pUCmini-Hyg-PKS (see example 2). To do so , pUCmini-Hyg-PKS was firstly cut by Asc I and filled in according to classical method then treated with Bsa I. The resulting plasmid pUCmini-Hyg-PKS-GFP contains all genetic elements permitting the production of recombinant GFP in Fusarium graminearum.

B) Transformation of Fusarium graminearum with pUCmini-Hyg-PKS-GFP and analysis of transformants

The transformation was done as described in example 3, wherein pUCmini-Hyg-PKS-GFP was linearized with EcoR47III. The correct integration of the plasmid in the PKS locus was observed after single conidiation by the absence of pigmentation of the recombinant mutants.

In addition, the integration was confirmed by PCR as described in example 4 using the following primer combinations EF-PKS (see example 4; SEQ ID NO:38) and ER-PKS (see example 4; SEQ ID NO:39), whereby no amplification were observed since the gene PKS is disrupted, whereas wild type strain or unspecific mutants presented a 714 bp DNA fragment corresponding to the expected PKS DNA fragment.

Using the primer combination EF-PKS (see example 4; SEQ ID NO:38) and

Lac 211 5' gcttctaatccgtactagtggatca 3' (SEQ ID NO 48)

the amplification of a 835 bp DNA corresponding to the 5' end plasmid integration in the PKS locus of the mutants was observed. No DNA fragment was amplified for the wildtype strain due the absence of the DNA sequence complementary to Lac211.

The primer combination

ANK 458 5' ctttgatcttttctacggggtctga 3' (SEQ ID NO 49) and

ER-PKS (see example 4; SEQ ID NO:39) led to the amplification of a 718 bp DNA corresponding to the 3' end plasmid integration in the PKS locus of the mutants. No DNA fragment was amplified for the wildtype strain due the absence of the DNA sequence complementary to ANK 458.

C) Detection of the production of GFP

The recombinant mutants were grown for a few days in CM-Hyg medium as described in example 3 except for glucose which was replaced by galactose as a carbon source. The fluorescence of GFP was detected using the polarstar spectrophotometer (Firma BMG; Ex: 385nm and Em: 520nm). In these conditions fluorescence was observed for the strains which showed integration of the plasmid whereas no fluorescence was observed for the wildtype strains.

Brief description of the figures

Figure 1: Map of pUCmini-Hyg

Figure 1: Map of PUCmini-Hyg TA

Claims

1. A plasmid vector for targeted transformation of filamentous fungi comprising

   a) an origin of replication for a host organism not originating from the filamentous fungi to be transformed;

   b) a selection marker for a host organism not originating from the filamentous fungi;

   c) a promotor facilitating recombinant expression in fungi that is functionally linked to the coding region of the hygromycin resistance gene which is functionally linked to a terminator which facilitates transcription termination in filamentous fungi;

   wherein the overall size of the elements a), b) and c) does not exceed 4500 bp; and

   d) a nucleic acid sequence which is homologous to nucleic acid sequences of the filamentous fungi to be transformed and makes homologous recombination in the filamentous fungi to be transformed possible.

2. A plasmid vector as claimed in claim 1, wherein the origin of replication a) originates from bacteria.

3. A plasmid vector as claimed in claims 1 to 2, wherein the selection marker b) imparts a resistance to antibiotics.

4. A plasmid vector according to claims 1 to 3, wherein the promotor of element c) is selected from the group consisting of the GPD-1-, PX6-, TEF-, CUP1-, PGK-, GAP1-, TPI, PHO5-, AOX1, GAL10/CYC-1, CYC1, OliC-, ADH-, TDH-, Kex2-, MFa- and the NMT-promotor.

5. A plasmid vector according to claims 1 to 4, wherein the terminator of element c) is selected from the group consisting of the AOX1-, nos-, PGK-, TrpC- and the CYC1-terminator.

6. A plasmid vector according to claims 1 to 5, wherein the promotor of element c) is the GPD-1-promotor and the terminator of element c) is the nos-terminator.

7. A plasmid vector according to claims 1 to 6, wherein the nucleic acid sequence d) is functionally linked to a promotor facilitating recombinant expression in filamentous fungi.

8. A plasmid vector according to claims 1 to 7, wherein the nucleic acid sequence d) is functionally linked to a transcription terminator facilitating recombinant expression in filamentous fungi.

9. A selection marker comprising a nucleic acid sequence encoding a polyketide synthetase fragment, wherein said nucleic acid sequence comprises

i. a nucleic acid sequence shown in SEQ ID NO:1 or SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5; or

ii. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:6 by back translation; or

iii. a functional equivalent of the nucleic acid sequence set forth in i) which is encoded by an amino acid sequence that has at least an identity of 50% with the SEQ ID NO: or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:6 that has at least an identity of 41% with the SEQ ID NO:6 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:8 that has at least an identity of 49% with the SEQ ID NO:8 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:10 that has at least an identity of 6; or

iv. parts of the nucleic acid sequence as defined in i., ii. or iii. consisting of at least 300bp; or

v. parts of the nucleic acid sequence as defined in i., ii. or iii. consisting of at least 300bp comprising

a) a nucleic acid sequence shown in SEQ ID NO:7 ; or

b) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:8 by back translation; or

c) a functional equivalent of a nucleic acid sequence set forth in a), which is encoded by amino acid sequence that has at least an identity of 85% with the SEQ ID NO:8.

10. Use of a nucleic acid sequence comprising

a) a nucleic acid sequence encoding a polyketide synthetase; or

b) parts of the nucleic acid sequence as defined in i. consisting of at least 300bp.

as marker for targeted transformation in filamentous fungi.

11. Use of a nucleic acid sequence according to claim 10 said nucleic acid sequence comprising

i. a nucleic acid sequence according to claim 9; or

ii. a nucleic acid sequence shown in SEQ ID NO:9 or SEQ ID NO: 11; or

iii. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:13 by back translation; or

iv. a functional equivalent of the nucleic acid sequence set forth in i), which is encoded by an amino acid sequence that has at least an identity of 40% with the SEQ ID NO:6 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:6 that has at least an identity of 38% with the SEQ ID NO:6 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:8 that has at least an identity of 39% with the SEQ ID NO:8 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:10 that has at least an identity of ; or

v. parts of the nucleic acid sequence as defined in ii., iii. or iv. consisting of at least 300bp; or

vi. parts of the nucleic acid sequence as definied in ii., iii or iv. consisting of at least 300bp comprising a nucleic acid sequence, which is encoded by an amino acid

sequence that has at least an identity of 68% with the SEQ ID NO:8.

12. A plasmid vector for targeted transformation of filamentous fungi additionally comprising a selection marker comprising a nucleic acid sequence encoding a polyketide synthetase fragment, said nucleic acid sequence comprising

i. a nucleic acid sequence according to claim 9; or

ii. a functional equivalent of the nucleic acid sequence set forth in i) which is encoded by an amino acid sequence that has at least an identity of 40% with the SEQ ID NO:6.

iii. a nucleic acid sequence shown in SEQ ID NO:9 or SEQ ID NO: 11;

iv. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:13 by back translation; or

v. parts of the nucleic acid sequence as defined in ii., iii. or iv. consisting of at least 300bp; or

vi. parts of the nucleic acid sequence as defined in i., ii. or iii. or iv. consisting of at least 300bp, which are encoded by an amino acid sequence that has at least an identity of 68% with SEQ ID NO:8.

13. A plasmid vector for targeted transformation of filamentous fungi as claimed in claims 1 to 8, additionally comprising a selection marker comprising a nucleic acid sequence encoding a polyketide synthetase fragment, said nucleic acid sequence comprising

i. a nucleic acid sequence according to claim 9; or

ii. a functional equivalent of the nucleic acid sequence set forth in i), which is encoded by an amino acid sequence that has at least an identity of 40% with the SEQ ID NO:6; or

iii. a nucleic acid sequence shown in SEQ ID NO:9 or SEQ ID NO: 11;

iv. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:13 by back translation; or

v. parts of the nucleic acid sequence as defined in ii., iii. or iv. consisting of at least 300bp; or

vi. parts of the nucleic acid sequence as defined in i., ii. or iii. or iv. consisting of at least 300bp comprising a nucleic acid sequence, which is encoded by a functional equivalent of an amino acid sequence that has at least an identity of 68% with the SEQ ID NO:8.

14. An expression cassette comprising

a) a promotor sequence in functional linkage with a nucleic acid sequence according to claim 9 in antisense orientation; and optionally

b) further genetic control sequences functionally linked to a nucleic acid sequence according to a).

15. A plasmid vector for targeted transformation of filamentous fungi additionally comprising an expression cassette according to claim 14.

16. A plasmid vector for targeted transformation of filamentous fungi as claimed in claims 1 to 8, additionally comprising an expression cassette according to claim 14.

17. A method for transforming filamentous fungi, comprising the following steps

a) transferring a plasmid vector according to claim 12, 13, 15 or 16 into a filamentous fungi;

b) selecting successfully transformed filamentous fungi by the absence of color.

18. An expression cassette comprising

a) a promotor sequence in functional linkage with a nucleic acid sequence comprising

i. a nucleic acid sequence shown in SEQ ID NO:3, 4 or 5; or

ii. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:6 by back translation; or

iii. a functional equivalent of the nucleic acid sequence set forth in i) which is encoded by an amino acid sequence shown in SEQ ID NO:6 that has at least an identity of 40% with the SEQ ID NO:6; or

iv. a nucleic acid sequence shown in SEQ ID NO:9 or SEQ ID NO: 11;

v. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:13 by back translation;

and optionally

b) further genetic control sequences functionally linked to a nucleic acid sequence according to a).

19. A method for transformation of filamentous fungi, comprising the following steps

a) providing a filamentous fungi, in which the polyketide synthetase gene is modified in such away that the polyketide synthetase cannot be functionally expressed;

b) transforming the filamentous fungi of step a) with an expression cassette according to claim 18 or a vector comprising the aforementioned expression cassette;

c) selecting successfully transformed filamentous fungi by the presence of color.

20. A method as claimed in claim 17 or 19, wherein the plasmid vector comprises at least an additional selection marker.

21. A method as claimed in claims 17, 19 or 20, wherein the selection is confirmed by PCR.

22. A method as claimed in claims 17, 19, 20 or 21, wherein the filamentous fungi are succesuflly transformed and identified in a high-throughput screening.

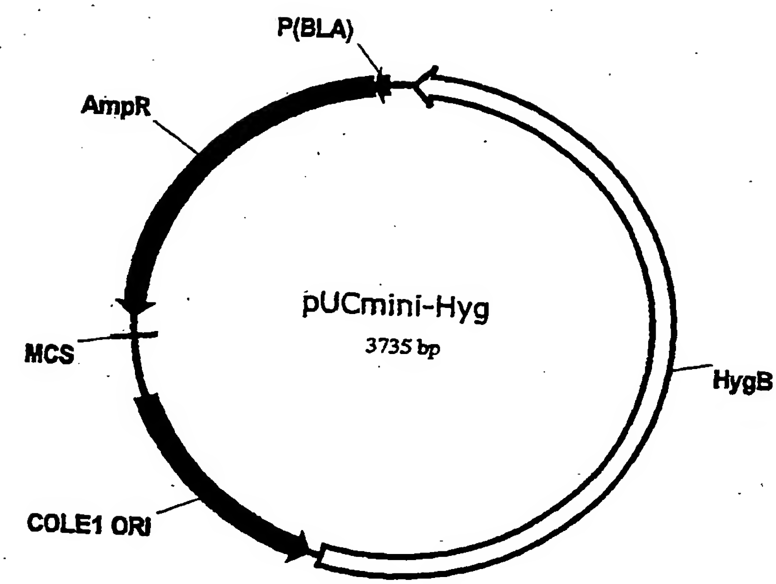

Figure 1

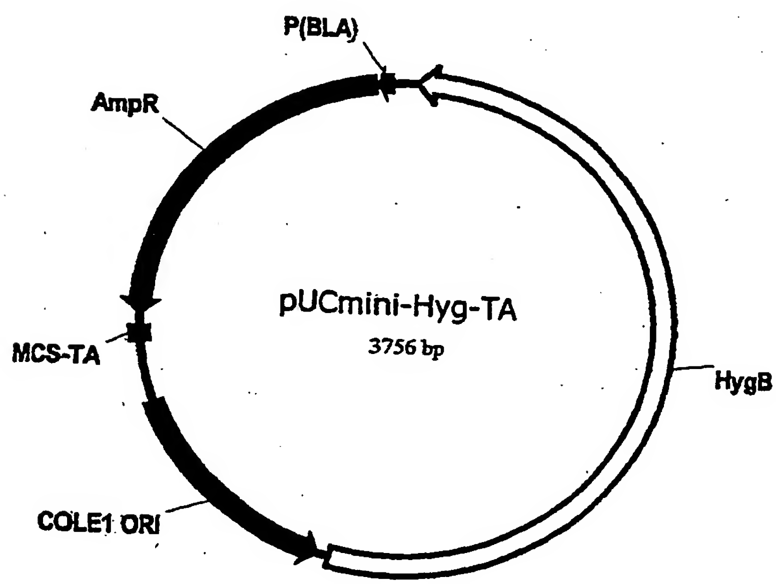

Figure 2

SEQUENCE LISTING

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Ile Ser Ser Leu Pro Ala Val Gln Gln Lys Leu Phe Pro Asn Phe Ala
50 55 60

gac att cag gaa ctc gtc tcc aag tca gat tgg ggc agt ggt aac cct 240
Asp Ile Gln Glu Leu Val Ser Lys Ser Asp Trp Gly Ser Gly Asn Pro
65 70 75 80

gct ttg aca agc gct tta gca tgc ttt tac cat ctt tgc agt ttc att 288
Ala Leu Thr Ser Ala Leu Ala Cys Phe Tyr His Leu Cys Ser Phe Ile
85 90 95

cac ttt tac gat gga caa ggt cgt acc ttt cct tcg gag aac agt cgc 336
His Phe Tyr Asp Gly Gln Gly Arg Thr Phe Pro Ser Glu Asn Ser Arg
100 105 110

att att gga ctt tgc gtt ggt tca ctc gct gct act gct gtc agt tgc 384
Ile Ile Gly Leu Cys Val Gly Ser Leu Ala Ala Thr Ala Val Ser Cys
115 120 125

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tcc aca tca ctg agt gaa ttg gta tca gct ggt gta gat gct gtt cgt    432
Ser Thr Ser Leu Ser Glu Leu Val Ser Ala Gly Val Asp Ala Val Arg
    130                 135                 140

gtg gca ttg cac gtc gga cta cgg gta tgg cga act acc tcc ctt ttc    480
Val Ala Leu His Val Gly Leu Arg Val Trp Arg Thr Thr Ser Leu Phe
145                 150                 155                 160

gat gta cca gac agg ccc tcc gcc act tgg ttc ata att gtg ccc gag    528
Asp Val Pro Asp Arg Pro Ser Ala Thr Trp Phe Ile Ile Val Pro Glu
                165                 170                 175

gca gta cta cca aga gaa tct gcg caa gac cga ctt gac tca ttc atc    576
Ala Val Leu Pro Arg Glu Ser Ala Gln Asp Arg Leu Asp Ser Phe Ile
            180                 185                 190

att gaa atg gga ctt gct cga tca tca gtt cct tac atc agc tcg gtc    624
Ile Glu Met Gly Leu Ala Arg Ser Ser Val Pro Tyr Ile Ser Ser Val
        195                 200                 205

gca cat cac aac atg acc atc agt ggt cca cca tcc gtc ctc gaa aag    672
Ala His His Asn Met Thr Ile Ser Gly Pro Pro Ser Val Leu Glu Lys
    210                 215                 220

ttc att cac agt ata tca aca tca ccg aaa gat tct ctt cca gtg ccg    720
Phe Ile His Ser Ile Ser Thr Ser Pro Lys Asp Ser Leu Pro Val Pro
225                 230                 235                 240

atc tat gct ccg tac cac gcc agc cat ctt tac agc atg gat gat gta    768
Ile Tyr Ala Pro Tyr His Ala Ser His Leu Tyr Ser Met Asp Asp Val
                245                 250                 255

gac gag gtc ctt agc ctg tct gca cct tct ttt gca tca gag tcc atc    816
Asp Glu Val Leu Ser Leu Ser Ala Pro Ser Phe Ala Ser Glu Ser Ile
            260                 265                 270

att cca ctc att tca agc tcc tcg ggt gac gag tta cag cca ctc aag    864
Ile Pro Leu Ile Ser Ser Ser Ser Gly Asp Glu Leu Gln Pro Leu Lys
        275                 280                 285

tat gca gat cta ctc cgc tgc tgt gtt agt gat atg ctc ata cag cca    912
Tyr Ala Asp Leu Leu Arg Cys Cys Val Ser Asp Met Leu Ile Gln Pro
    290                 295                 300

ctg gat ctt acc aag gtc tca caa gca gtg gcc cag ctt ctc gag gtt    960
Leu Asp Leu Thr Lys Val Ser Gln Ala Val Ala Gln Leu Leu Glu Val
305                 310                 315                 320

agc tca tct aca cgt gcc ata ata aag cct ata gca acc agc gtc tcc    1008
Ser Ser Ser Thr Arg Ala Ile Ile Lys Pro Ile Ala Thr Ser Val Ser
                325                 330                 335

aac agt cta gtg tct gtt ttg gag ccg acg cta gca gaa cga tgc gcc    1056
Asn Ser Leu Val Ser Val Leu Glu Pro Thr Leu Ala Glu Arg Cys Ala
            340                 345                 350
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gtg gac aac agc atg ggg ccc aaa gcc tcg acc agc cac tca tca gca 1104
Val Asp Asn Ser Met Gly Pro Lys Ala Ser Thr Ser His Ser Ser Ala
355 360 365

gag aca caa acc gag tca tca agc aag aac tcc aaa att gcg att gtt 1152
Glu Thr Gln Thr Glu Ser Ser Ser Lys Asn Ser Lys Ile Ala Ile Val
370 375 380

gct atg tct ggt cgc ttt cca gac gca gct gac ttg agt gaa ttc tgg 1200
Ala Met Ser Gly Arg Phe Pro Asp Ala Ala Asp Leu Ser Glu Phe Trp
385 390 395 400

gat ctt ctc tac gaa ggt cgc gat gtt cat cga caa att ccc gag gac 1248
Asp Leu Leu Tyr Glu Gly Arg Asp Val His Arg Gln Ile Pro Glu Asp
405 410 415

cga ttc aac gca gag ctc cat tac gac gct act ggg cga cgt aag aac 1296
Arg Phe Asn Ala Glu Leu His Tyr Asp Ala Thr Gly Arg Arg Lys Asn
420 425 430

acc agc aag gtc atg aat ggc tgc ttc atc aag gaa cca gga ctg ttc 1344
Thr Ser Lys Val Met Asn Gly Cys Phe Ile Lys Glu Pro Gly Leu Phe
435 440 445

gac gct agg ttc ttc aac atg tct cca aag gaa gct gag cag tcg gat 1392
Asp Ala Arg Phe Phe Asn Met Ser Pro Lys Glu Ala Glu Gln Ser Asp
450 455 460

cct ggc cag cga atg gcc ctc gaa aca gct tac gag gcg ctt gag atg 1440
Pro Gly Gln Arg Met Ala Leu Glu Thr Ala Tyr Glu Ala Leu Glu Met
465 470 475 480

gct agt atc gta cca gac aga aca cct tcg aca cag aga gat cgt gtt 1488
Ala Ser Ile Val Pro Asp Arg Thr Pro Ser Thr Gln Arg Asp Arg Val
485 490 495

ggt gtg ttc tac ggc atg act agc gat gat tgg aga gag gtc aac agt 1536
Gly Val Phe Tyr Gly Met Thr Ser Asp Asp Trp Arg Glu Val Asn Ser
500 505 510

ggg cag aat gtc gac act tat ttt att cct ggt ggc aac aga gcg ttc 1584
Gly Gln Asn Val Asp Thr Tyr Phe Ile Pro Gly Gly Asn Arg Ala Phe
515 520 525

act cct ggt cga ctc aac tac ttc ttc aag ttc agt ggg cct agc gct 1632
Thr Pro Gly Arg Leu Asn Tyr Phe Phe Lys Phe Ser Gly Pro Ser Ala
530 535 540

agt gtt gat acg gct tgc tcc tcc agt ctc gtt ggc ttg cac ttg gct 1680
Ser Val Asp Thr Ala Cys Ser Ser Ser Leu Val Gly Leu His Leu Ala
545 550 555 560

tgt aat tcc ctc tgg aga aat gat tgc gat aca gct att gcg ggc gga 1728
Cys Asn Ser Leu Trp Arg Asn Asp Cys Asp Thr Ala Ile Ala Gly Gly
565 570 575

acc aat gtc atg act aac cct gac aac ttc gct ggt ttg gac cga ggc 1776
Thr Asn Val Met Thr Asn Pro Asp Asn Phe Ala Gly Leu Asp Arg Gly
580 585 590

cac ttc cta tct aga acc ggc aac tgc aac acc ttt gac gat gga gca 1824
His Phe Leu Ser Arg Thr Gly Asn Cys Asn Thr Phe Asp Asp Gly Ala
595 600 605

gac gga tac tgt cga gct gat ggc gtc gga acc atc atc ctc aag cgg 1872
Asp Gly Tyr Cys Arg Ala Asp Gly Val Gly Thr Ile Ile Leu Lys Arg
610 615 620

ctt gag gac gcc gaa gct gac aat gac cct att ctc ggt gtc att ctg 1920
Leu Glu Asp Ala Glu Ala Asp Asn Asp Pro Ile Leu Gly Val Ile Leu
625 630 635 640

ggc gct tac aca aac cac tca gcc gaa gca gta tcc atc act cga cca 1968
Gly Ala Tyr Thr Asn His Ser Ala Glu Ala Val Ser Ile Thr Arg Pro
645 650 655

cat gcc gga gct caa gag tac atc ttc tcc aaa ctc ctc cgt gag tcg 2016
His Ala Gly Ala Gln Glu Tyr Ile Phe Ser Lys Leu Leu Arg Glu Ser
660 665 670

ggc acc gat ccc tac aac gtt agc tac atc gag atg cac ggc aca ggc 2064
Gly Thr Asp Pro Tyr Asn Val Ser Tyr Ile Glu Met His Gly Thr Gly
675 680 685

act caa gcc ggc gac gca acc gag atg aca tcc gtc ctc aag acg ttt 2112
Thr Gln Ala Gly Asp Ala Thr Glu Met Thr Ser Val Leu Lys Thr Phe
690 695 700

gct cct acc agc ggc ttc ggc ggt cga ttg cct cac caa aac ctt cac 2160
Ala Pro Thr Ser Gly Phe Gly Gly Arg Leu Pro His Gln Asn Leu His
705 710 715 720

ttg ggt tca gtc aag gcc aat gtc ggg cac ggt gaa tcc gca tct ggt 2208
Leu Gly Ser Val Lys Ala Asn Val Gly His Gly Glu Ser Ala Ser Gly
725 730 735

atc att gct ctg atc aag acg ctg ctt atg atg gag aag aac atg atc 2256
Ile Ile Ala Leu Ile Lys Thr Leu Leu Met Met Glu Lys Asn Met Ile
740 745 750

ccg ccg cat tgt ggt atc aag aca aag atc aat cac cat ttt cct acg 2304
Pro Pro His Cys Gly Ile Lys Thr Lys Ile Asn His His Phe Pro Thr
755 760 765

gat ctc act cag cgc aat gtc cat atc gcc aaa gtt ccg aca tct tgg 2352
Asp Leu Thr Gln Arg Asn Val His Ile Ala Lys Val Pro Thr Ser Trp
770 775 780

aca aga tcg ggt caa gcc aat cca cgc att gct ttc gtc aat aac ttc 2400
Thr Arg Ser Gly Gln Ala Asn Pro Arg Ile Ala Phe Val Asn Asn Phe
785 790 795 800

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tct gcc gct ggt ggt aac tct gct gtc cta ctg caa gat gct cct cag     2448
Ser Ala Ala Gly Gly Asn Ser Ala Val Leu Leu Gln Asp Ala Pro Gln
                805                 810                 815

cca tcg gta gtt tcg gat gtt aca gac cct cgc aca tcc cat gtt gtc     2496
Pro Ser Val Val Ser Asp Val Thr Asp Pro Arg Thr Ser His Val Val
            820                 825                 830

act atg tcc gct cga tca gca gat tcc ctc agg aag aac ctc gcc aat     2544
Thr Met Ser Ala Arg Ser Ala Asp Ser Leu Arg Lys Asn Leu Ala Asn
        835                 840                 845

ctc aag gag ctt gta gaa ggc caa ggt gac tcg gag gtc ggc ttc ctg     2592
Leu Lys Glu Leu Val Glu Gly Gln Gly Asp Ser Glu Val Gly Phe Leu
    850                 855                 860

agc aag ctg tcc tac aca acc acc gcc agg cgc atg cat cat caa ttc     2640
Ser Lys Leu Ser Tyr Thr Thr Thr Ala Arg Arg Met His His Gln Phe
865                 870                 875                 880

cga gct tcg gtc aca gca cag act cgt gaa cag ctg ctg aag ggc ctt     2688
Arg Ala Ser Val Thr Ala Gln Thr Arg Glu Gln Leu Leu Lys Gly Leu
                885                 890                 895

gat tcc gcc att gaa cgc cag gat gtg aag agg atc ccc gcc gcc gcg     2736
Asp Ser Ala Ile Glu Arg Gln Asp Val Lys Arg Ile Pro Ala Ala Ala
            900                 905                 910

ccc tct gtc ggc ttt gtg ttt agc ggc caa ggc gcc caa tac cgt ggt     2784
Pro Ser Val Gly Phe Val Phe Ser Gly Gln Gly Ala Gln Tyr Arg Gly
        915                 920                 925

atg ggc aag gag tac ttt aca tct ttc aca gcc ttc cgc tct gag atc     2832
Met Gly Lys Glu Tyr Phe Thr Ser Phe Thr Ala Phe Arg Ser Glu Ile
    930                 935                 940

atg tct tac gac agt atc gcc caa gcc caa ggc ttc ccg tca atc ctc     2880
Met Ser Tyr Asp Ser Ile Ala Gln Ala Gln Gly Phe Pro Ser Ile Leu
945                 950                 955                 960

cca ctg atc cga gga gag gtg gaa gct gac tcg ttg agt cct gtt gag     2928
Pro Leu Ile Arg Gly Glu Val Glu Ala Asp Ser Leu Ser Pro Val Glu
                965                 970                 975

atc cag ctg ggt ctc act tgc ctg cag atg gca ctg gcc aag cta tgg     2976
Ile Gln Leu Gly Leu Thr Cys Leu Gln Met Ala Leu Ala Lys Leu Trp
            980                 985                 990

aag tca ttc ggt gtt gag cca ggc ttt gtt ctc gga cac agc tta ggc     3024
Lys Ser Phe Gly Val Glu Pro Gly Phe Val Leu Gly His Ser Leu Gly
        995                 1000                1005

cac tat gct gct tta cac gtc gct ggt gtt ctg tcc gcc aat gat acc     3072
His Tyr Ala Ala Leu His Val Ala Gly Val Leu Ser Ala Asn Asp Thr
    1010                1015                1020
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att tac ctc act ggc atc aga gcg cag ctg ctc gtg gat aag tgc cag    3120
Ile Tyr Leu Thr Gly Ile Arg Ala Gln Leu Leu Val Asp Lys Cys Gln
1025                1030                1035                1040

gca gga acc cac tca atg ctg gca gtg agg gca tcc tta cta cag atc    3168
Ala Gly Thr His Ser Met Leu Ala Val Arg Ala Ser Leu Leu Gln Ile
                1045                1050                1055

caa cag ttc ctc gat gcc aac att cac gag gtt gca tgt gtc aat ggt    3216
Gln Gln Phe Leu Asp Ala Asn Ile His Glu Val Ala Cys Val Asn Gly
            1060                1065                1070

tca cga gaa gtc gtc atc agt gga cgc gtt gcc gac att gac cag ctg    3264
Ser Arg Glu Val Val Ile Ser Gly Arg Val Ala Asp Ile Asp Gln Leu
        1075                1080                1085

gtt ggc ctt ttg tcg gct gac aac atc aag gcg acc cgc gtc aag gtg    3312
Val Gly Leu Leu Ser Ala Asp Asn Ile Lys Ala Thr Arg Val Lys Val
    1090                1095                1100

cca ttt gcc ttc cac tca gcg cag gtt gac ccc att ctc tcc gac ttg    3360
Pro Phe Ala Phe His Ser Ala Gln Val Asp Pro Ile Leu Ser Asp Leu
1105                1110                1115                1120

gat aca gcg gcg tcg cgc gtc acc ttc cac tcc ctc cag att cct gtt    3408
Asp Thr Ala Ala Ser Arg Val Thr Phe His Ser Leu Gln Ile Pro Val
                1125                1130                1135

ctt tgt gcc ctt gac agc tct gtc atc agc cct gga aac cat ggt gtc    3456
Leu Cys Ala Leu Asp Ser Ser Val Ile Ser Pro Gly Asn His Gly Val
            1140                1145                1150

att ggt ccc ctt cat cta cag cga cat tgt cgt gag aca gtc aac ttt    3504
Ile Gly Pro Leu His Leu Gln Arg His Cys Arg Glu Thr Val Asn Phe
        1155                1160                1165

gag ggt gct cta cat gct gcg gag cac gag aag atc atc aac aag aca    3552
Glu Gly Ala Leu His Ala Ala Glu His Glu Lys Ile Ile Asn Lys Thr
    1170                1175                1180

tca act cta tgg atc gag att ggt ccc cat gtt gtc tgc tct acc ttc    3600
Ser Thr Leu Trp Ile Glu Ile Gly Pro His Val Val Cys Ser Thr Phe
1185                1190                1195                1200

ctc aag tcc agc ctt ggt cca agc aca cct gct atc gca tcg ctt cgc    3648
Leu Lys Ser Ser Leu Gly Pro Ser Thr Pro Ala Ile Ala Ser Leu Arg
                1205                1210                1215

cga aat gac gat tgc tgg aag gtg ttg gct gat ggt ttg agc agt ctc    3696
Arg Asn Asp Asp Cys Trp Lys Val Leu Ala Asp Gly Leu Ser Ser Leu
            1220                1225                1230

tac agc agc ggg ttg aca att gac ttg aac gag tat cat cgc gac ttc    3744
Tyr Ser Ser Gly Leu Thr Ile Asp Leu Asn Glu Tyr His Arg Asp Phe
        1235                1240                1245
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aag gcc tct cac cag gta ctt cgt ctg cct tgt tac agc tgg gag cac 3792
Lys Ala Ser His Gln Val Leu Arg Leu Pro Cys Tyr Ser Trp Glu His
1250 1255 1260

aag aat tac tgg ata cag tac aag tac gat tgg tcc ttg gct aaa ggt 3840
Lys Asn Tyr Trp Ile Gln Tyr Lys Tyr Asp Trp Ser Leu Ala Lys Gly
1265 1270 1275 1280

gat cct cca att gcc cct aac agc tcg gtt gaa gca gtc tca gct tta 3888
Asp Pro Pro Ile Ala Pro Asn Ser Ser Val Glu Ala Val Ser Ala Leu
1285 1290 1295

tca aca ccc tcg gtc cag aag att cta cag gag act tcc ctt gat cag 3936
Ser Thr Pro Ser Val Gln Lys Ile Leu Gln Glu Thr Ser Leu Asp Gln
1300 1305 1310

gta ttg act atc gtt gct gag aca gat ctc gcg agc cct cta ttg tca 3984
Val Leu Thr Ile Val Ala Glu Thr Asp Leu Ala Ser Pro Leu Leu Ser
1315 1320 1325

gag gtt gcc caa ggt cat cgg gtc aac ggt gtc aaa gtc tgc aca tct 4032
Glu Val Ala Gln Gly His Arg Val Asn Gly Val Lys Val Cys Thr Ser
1330 1335 1340

tcc gtg tac gct gat gtt ggc ttg acg ctg ggt aag tac att ttg gac 4080
Ser Val Tyr Ala Asp Val Gly Leu Thr Leu Gly Lys Tyr Ile Leu Asp
1345 1350 1355 1360

aac tac cgc acc gac tta gag ggt tat gcg gtc gat gtt cac ggt att 4128
Asn Tyr Arg Thr Asp Leu Glu Gly Tyr Ala Val Asp Val His Gly Ile
1365 1370 1375

gag gtc cac aag cca ctt ctt ctc aaa gaa gac atg aac gga acg ccc 4176
Glu Val His Lys Pro Leu Leu Leu Lys Glu Asp Met Asn Gly Thr Pro
1380 1385 1390

cag gct aca ccg ttc cgc atc gaa gtg cga tac cca atc cag agc acc 4224
Gln Ala Thr Pro Phe Arg Ile Glu Val Arg Tyr Pro Ile Gln Ser Thr
1395 1400 1405

acg gcg ctg atg agc atc tcc acc act ggc ccc aac ggt cag cac atc 4272
Thr Ala Leu Met Ser Ile Ser Thr Thr Gly Pro Asn Gly Gln His Ile
1410 1415 1420

aag cat gct aac tgc gaa ctt cga ctc gag cat ccg tcg caa tgg gaa 4320
Lys His Ala Asn Cys Glu Leu Arg Leu Glu His Pro Ser Gln Trp Glu
1425 1430 1435 1440

gcg gag tgg gat cgc caa gcc tac ctc atc aat cgc agc gtc aac tgc 4368
Ala Glu Trp Asp Arg Gln Ala Tyr Leu Ile Asn Arg Ser Val Asn Cys
1445 1450 1455

ctt ctg cag cga tca gca caa ggt ttg gac agc atg ttg gca acc gga 4416
Leu Leu Gln Arg Ser Ala Gln Gly Leu Asp Ser Met Leu Ala Thr Gly
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atg gtc tac aag gtc ttc tcc tcc ctc gtc gac tat gcc gat ggc tac 4464
Met Val Tyr Lys Val Phe Ser Ser Leu Val Asp Tyr Ala Asp Gly Tyr
1475 1480 1485

aag ggt ctg cag gag gtt gtc ttg cac agc caa gag ctc gag ggc aca 4512
Lys Gly Leu Gln Glu Val Val Leu His Ser Gln Glu Leu Glu Gly Thr
1490 1495 1500

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Ala Lys Val Arg Phe Gln Thr Pro Ser Gly Gly Phe Val Cys Asn Pro
1505 1510 1515 1520

atg tgg att gac agc tgt ggt cag acg acc ggc ttc atg atg aac tgt 4608
Met Trp Ile Asp Ser Cys Gly Gln Thr Thr Gly Phe Met Met Asn Cys
1525 1530 1535

cat cag act acg ccc aat gac tac gtc tac gtc aat cat ggc tgg aag 4656
His Gln Thr Thr Pro Asn Asp Tyr Val Tyr Val Asn His Gly Trp Lys
1540 1545 1550

tcg atg aga ttg gcc aag gcg ttc cgt gaa gat ggt acc tat cgg act 4704
Ser Met Arg Leu Ala Lys Ala Phe Arg Glu Asp Gly Thr Tyr Arg Thr
1555 1560 1565

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Tyr Ile Arg Met Arg Pro Ile Asp Ser Thr Lys Phe Ala Gly Asp Leu
1570 1575 1580

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Tyr Ile Leu Asp Glu Asp Asp Thr Val Val Gly Val Tyr Gly Asp Ile
1585 1590 1595 1600

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Thr Phe Gln Gly Leu Pro Arg Arg Val Leu Asn Thr Val Leu Pro Ser
1605 1610 1615

gcc aac gcg gtt cca gtt gat gct ccc atg ggt cga cgg gac gtg cct 4896
Ala Asn Ala Val Pro Val Asp Ala Pro Met Gly Arg Arg Asp Val Pro
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Pro Ser Arg Met Asp Val Pro Pro Val Arg Ser Gly Glu Gly Pro Pro
1635 1640 1645

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1650 1655 1660

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1665 1670 1675 1680

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Glu Ile Gly Leu Gly Leu Asp Val Leu Ser Asp Asp Glu Leu Asp Phe
1685 1690 1695

gcg gac cac ggt gtc gac tca ctc ctc tca ttg acc atc act ggt cgc 5136
Ala Asp His Gly Val Asp Ser Leu Leu Ser Leu Thr Ile Thr Gly Arg
1700 1705 1710

atg cgt gag gaa ttg ggt ctc gac gtt gaa tct aca gca ttc atg aac 5184
Met Arg Glu Glu Leu Gly Leu Asp Val Glu Ser Thr Ala Phe Met Asn
1715 1720 1725

tgt ccc act ttg ggc agc ttt aaa ttg ttc cta gga ctt gtc gat cag 5232
Cys Pro Thr Leu Gly Ser Phe Lys Leu Phe Leu Gly Leu Val Asp Gln
1730 1735 1740

gac aat aag ggc agc agc ggc agt gat ggc agt ggt agg agc agt cca 5280
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1745 1750 1755 1760

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1765 1770 1775

gac cag gac aag ata gtc agc agt cac tcg ctt cac cag ttc caa gcc 5376
Asp Gln Asp Lys Ile Val Ser Ser His Ser Leu His Gln Phe Gln Ala
1780 1785 1790

agt tcg acg ctt cta cag ggc agt ccc agt aaa gct cgc tcg act ttg 5424
Ser Ser Thr Leu Leu Gln Gly Ser Pro Ser Lys Ala Arg Ser Thr Leu
1795 1800 1805

ttc ttg cta cca gat ggc tcg gga tct gcc aca tcc tac gct tcc ctt 5472
Phe Leu Leu Pro Asp Gly Ser Gly Ser Ala Thr Ser Tyr Ala Ser Leu
1810 1815 1820

ccc ccg atc tct cca gac gga gat gtt gct gtc tac ggg ttg aac tgt 5520
Pro Pro Ile Ser Pro Asp Gly Asp Val Ala Val Tyr Gly Leu Asn Cys
1825 1830 1835 1840

cca tgg ctg aag gac tct agt tac ctc gtc gag ttt gga ctc aag ggc 5568
Pro Trp Leu Lys Asp Ser Ser Tyr Leu Val Glu Phe Gly Leu Lys Gly
1845 1850 1855

ttg aca gag ctc tat gtc aac gag ata ctc cgt cgc aag cca cag ggt 5616
Leu Thr Glu Leu Tyr Val Asn Glu Ile Leu Arg Arg Lys Pro Gln Gly
1860 1865 1870

cct tac aat ttg gga gga tgg tca gcc ggt ggc att tgc gct tat gaa 5664
Pro Tyr Asn Leu Gly Gly Trp Ser Ala Gly Gly Ile Cys Ala Tyr Glu
1875 1880 1885

gct gcc ctg atc ctc acc aga gca gga cac caa gtc gat cgc ctt atc 5712
Ala Ala Leu Ile Leu Thr Arg Ala Gly His Gln Val Asp Arg Leu Ile
1890 1895 1900

ttg att gac tct ccc aat ccc gtt ggt ctt gag aag cta cct cct cgc 5760
Leu Ile Asp Ser Pro Asn Pro Val Gly Leu Glu Lys Leu Pro Pro Arg
1905 1910 1915 1920

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Leu Tyr Asp Phe Leu Asn Ser Gln Asn Val Phe Gly Ser Asp Asn Pro
1925 1930 1935

cac agc act gct gga aca agc gtc aaa gct cca gaa tgg ctt ctt gca 5856
His Ser Thr Ala Gly Thr Ser Val Lys Ala Pro Glu Trp Leu Leu Ala
1940 1945 1950

cat ttc ctg gcc ttc att gac gct ctg gat gct tat gtc gca gtg cct 5904
His Phe Leu Ala Phe Ile Asp Ala Leu Asp Ala Tyr Val Ala Val Pro
1955 1960 1965

tgg gac tct ggt cta gtc ggt cta gca tca ccg ctc cct gca ccg ccg 5952
Trp Asp Ser Gly Leu Val Gly Leu Ala Ser Pro Leu Pro Ala Pro Pro
1970 1975 1980

cag aca tac atg ctg tgg gca gaa gac gga gtt tgc aaa gac tct gat 6000
Gln Thr Tyr Met Leu Trp Ala Glu Asp Gly Val Cys Lys Asp Ser Asp
1985 1990 1995 2000

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Ser Ala Arg Pro Glu Tyr Arg Asp Asp Asp Pro Arg Glu Met Arg Trp
2005 2010 2015

ctg ttg gag aac aga aca aac ttt ggt ccg aat ggt tgg gag gcg cta 6096
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2020 2025 2030

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2035 2040 2045

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His Phe Ser Met Leu Lys Arg Gly Arg Asn Ala Glu Tyr Val Ser Ala
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Ala Leu Thr Ser Ala Leu Ala Cys Phe Tyr His Leu Cys Ser Phe Ile
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His Phe Tyr Asp Gly Gln Gly Arg Thr Phe Pro Ser Glu Asn Ser Arg
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Ala Val Leu Pro Arg Glu Ser Ala Gln Asp Arg Leu Asp Ser Phe Ile
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Phe Ile His Ser Ile Ser Thr Ser Pro Lys Asp Ser Leu Pro Val Pro
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Ile Pro Leu Ile Ser Ser Ser Ser Gly Asp Glu Leu Gln Pro Leu Lys
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Tyr Ala Asp Leu Leu Arg Cys Cys Val Ser Asp Met Leu Ile Gln Pro
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Leu Asp Leu Thr Lys Val Ser Gln Ala Val Ala Gln Leu Leu Glu Val
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<211> 2109
<212> PRT
<213> Aspergillus parasiticus

<400> 12

Met Ala Gln Ser Arg Gln Leu Phe Leu Phe Gly Asp Gln Thr Ala Asp
1 5 10 15

Phe Val Pro Lys Leu Arg Ser Leu Leu Ser Val Gln Asp Ser Pro Ile
20 25 30

Leu Ala Ala Phe Leu Asp Gln Ser His Tyr Val Val Arg Ala Gln Met
35 40 45

Leu Gln Ser Met Asn Thr Val Asp His Lys Leu Ala Arg Thr Ala Asp
50 55 60

Leu Arg Gln Met Val Gln Lys Tyr Val Asp Gly Lys Leu Thr Pro Ala
65 70 75 80

Phe Arg Thr Ala Leu Val Cys Leu Cys Gln Leu Gly Cys Phe Ile Arg
85 90 95

Glu Tyr Glu Glu Ser Gly Asn Met Tyr Pro Gln Pro Ser Asp Ser Tyr
100 105 110

Val Leu Gly Phe Cys Met Gly Ser Leu Ala Ala Val Ala Val Ser Cys
115 120 125

Ser Arg Ser Leu Ser Glu Leu Leu Pro Ile Ala Val Gln Thr Val Leu
130 135 140

Ile Ala Phe Arg Leu Gly Leu Cys Ala Leu Glu Met Arg Asp Arg Val
145 150 155 160

Asp Gly Cys Ser Asp Asp Arg Gly Asp Pro Trp Ser Thr Ile Val Trp
165 170 175

Gly Leu Asp Pro Gln Gln Ala Arg Asp Gln Ile Glu Val Phe Cys Arg
180 185 190

Thr Thr Asn Val Pro Gln Thr Arg Arg Pro Trp Ile Ser Cys Ile Ser
195 200 205

Lys Asn Ala Ile Thr Leu Ser Gly Ser Pro Ser Thr Leu Arg Ala Phe
210 215 220

Cys Ala Met Pro Gln Met Ala Gln His Arg Thr Ala Pro Ile Pro Ile
225 230 235 240

Cys Leu Pro Ala His Asn Gly Ala Leu Phe Thr Gln Ala Asp Ile Thr
245 250 255

Thr Ile Leu Asp Thr Thr Pro Thr Thr Pro Trp Glu Gln Leu Pro Gly
260 265 270

Gln Ile Pro Tyr Ile Ser His Val Thr Gly Asn Val Val Gln Thr Ser
275 280 285

Asn Tyr Arg Asp Leu Ile Glu Val Ala Leu Ser Glu Thr Leu Leu Glu
290 295 300

Gln Val Arg Leu Asp Leu Val Glu Thr Gly Leu Pro Arg Leu Leu Gln
305 310 315 320

Ser Arg Gln Val Lys Ser Val Thr Ile Val Pro Phe Leu Thr Arg Met
325 330 335

Asn Glu Thr Met Ser Asn Ile Leu Pro Asp Ser Phe Ile Ser Thr Glu
340 345 350

Thr Arg Thr Asp Thr Gly Arg Ala Ile Pro Ala Ser Gly Arg Pro Gly
355 360 365

Ala Gly Lys Cys Lys Leu Ala Ile Val Ser Met Ser Gly Arg Phe Pro
370 375 380

Glu Ser Pro Thr Thr Glu Ser Phe Trp Asp Leu Leu Tyr Lys Gly Leu
385 390 395 400

Asp Val Cys Lys Glu Val Pro Arg Arg Arg Trp Asp Ile Asn Thr His
405 410 415

Val Asp Pro Ser Gly Lys Ala Arg Asn Lys Gly Ala Thr Lys Trp Gly
420 425 430

Cys Trp Leu Asp Phe Ser Gly Asp Phe Asp Pro Arg Phe Phe Gly Ile
435 440 445

Ser Pro Lys Glu Ala Pro Gln Met Asp Pro Ala Gln Arg Met Ala Leu
450 455 460

Met Ser Thr Tyr Glu Ala Met Glu Arg Ala Gly Leu Val Pro Asp Thr
465 470 475 480

Thr Pro Ser Thr Gln Arg Asp Arg Ile Gly Val Phe His Gly Val Thr
485 490 495

Ser Asn Asp Trp Met Glu Thr Asn Thr Ala Gln Asn Ile Asp Thr Tyr
500 505 510

Phe Ile Thr Gly Gly Asn Arg Gly Phe Ile Pro Gly Arg Ile Asn Phe
515 520 525

Cys Phe Glu Phe Ala Gly Pro Ser Tyr Thr Asn Asp Thr Ala Cys Ser
530 535 540

Ser Ser Leu Ala Ala Ile His Leu Ala Cys Asn Ser Leu Trp Arg Gly
545 550 555 560

Asp Cys Asp Thr Ala Val Ala Gly Gly Thr Asn Met Ile Tyr Thr Pro
565 570 575

Asp Gly His Thr Gly Leu Asp Lys Gly Phe Phe Leu Ser Arg Thr Gly
580 585 590

Asn Cys Lys Pro Tyr Asp Asp Lys Ala Asp Gly Tyr Cys Arg Ala Glu
595 600 605

Gly Val Gly Thr Val Phe Ile Lys Arg Leu Glu Asp Ala Leu Ala Asp
610 615 620

Asn Asp Pro Ile Leu Gly Val Ile Leu Asp Ala Lys Thr Asn His Ser
625 630 635 640

Ala Met Ser Glu Ser Met Thr Arg Pro His Val Gly Ala Gln Ile Asp
645 650 655

Asn Met Thr Ala Ala Leu Asn Thr Thr Gly Leu His Pro Asn Asp Phe
660 665 670

Ser Tyr Ile Glu Met His Gly Thr Gly Thr Gln Val Gly Asp Ala Val
675 680 685

Glu Met Glu Ser Val Leu Ser Val Phe Ala Pro Ser Glu Thr Ala Arg
690 695 700

Lys Ala Asp Gln Pro Leu Phe Val Gly Ser Ala Lys Ala Asn Val Gly
705 710 715 720

His Gly Glu Gly Val Ser Gly Val Thr Ser Leu Ile Lys Val Leu Met
725 730 735

Met Met Gln His Asp Thr Ile Pro Pro His Cys Gly Ile Lys Pro Gly
740 745 750

Ser Lys Ile Asn Arg Asn Phe Pro Asp Leu Gly Ala Arg Asn Val His
755 760 765

Ile Ala Phe Glu Pro Lys Pro Trp Pro Arg Thr His Thr Pro Arg Arg
770 775 780

Val Leu Ile Asn Asn Phe Ser Ala Ala Gly Gly Asn Thr Ala Leu Ile
785 790 795 800

Val Glu Asp Ala Pro Glu Arg His Trp Pro Thr Glu Lys Asp Pro Arg
805 810 815

Ser Ser His Ile Val Ala Leu Ser Ala His Val Gly Ala Ser Met Lys
820 825 830

Thr Asn Leu Glu Arg Leu His Gln Tyr Leu Leu Lys Asn Pro His Thr
835 840 845

Asp Leu Ala Gln Leu Ser Tyr Thr Thr Thr Ala Arg Arg Trp His Tyr
850 855 860

Leu His Arg Val Ser Val Thr Gly Ala Ser Val Glu Glu Val Thr Arg
865 870 875 880

Lys Leu Glu Met Ala Ile Gln Asn Gly Asp Gly Val Ser Arg Pro Lys
885 890 895

Ser Lys Pro Lys Ile Leu Phe Ala Phe Thr Gly Gln Gly Ser Gln Tyr
900 905 910

Ala Thr Met Gly Lys Gln Val Tyr Asp Ala Tyr Pro Ser Phe Arg Glu
915 920 925

Asp Leu Glu Lys Phe Asp Arg Leu Ala Gln Ser His Gly Phe Pro Ser
930 935 940

Phe Leu His Val Cys Thr Ser Pro Lys Gly Asp Val Glu Glu Met Ala
945 950 955 960

Pro Val Val Val Gln Leu Ala Ile Thr Cys Leu Gln Met Ala Leu Thr
965 970 975

Asn Leu Met Thr Ser Phe Gly Ile Arg Pro Asp Val Thr Val Gly His
980 985 990

Ser Leu Gly Glu Phe Ala Ala Leu Tyr Ala Ala Gly Val Leu Ser Ala
995 1000 1005

Ser Asp Val Val Tyr Leu Val Gly Gln Arg Ala Glu Leu Leu Gln Glu
1010 1015 1020

Arg Cys Gln Arg Gly Thr His Ala Met Leu Ala Val Lys Ala Thr Pro
1025 1030 1035 1040

Glu Ala Leu Ser Gln Trp Ile Gln Asp His Asp Cys Glu Val Ala Cys
1045 1050 1055

Ile Asn Gly Pro Glu Asp Thr Val Leu Ser Gly Thr Thr Lys Asn Val
1060 1065 1070

Ala Glu Val Gln Arg Ala Met Thr Asp Asn Gly Ile Lys Cys Thr Leu
1075 1080 1085

Leu Lys Leu Pro Phe Ala Phe His Ser Ala Gln Val Gln Pro Ile Leu
1090 1095 1100

Asp Asp Phe Glu Ala Leu Ala Gln Gly Ala Thr Phe Ala Lys Pro Gln
1105 1110 1115 1120

Leu Leu Ile Leu Ser Pro Leu Leu Arg Thr Glu Ile His Glu Gln Gly
1125 1130 1135

Val Val Thr Pro Ser Tyr Val Ala Gln His Cys Arg His Thr Val Asp
1140 1145 1150

Met Ala Gln Ala Leu Arg Ser Ala Arg Glu Lys Gly Leu Ile Asp Asp
1155 1160 1165

Lys Thr Leu Val Ile Glu Leu Gly Pro Lys Pro Leu Ile Ser Gly Met
1170 1175 1180

Val Lys Met Thr Leu Gly Asp Lys Ile Ser Thr Leu Pro Thr Leu Ala
1185 1190 1195 1200

Pro Asn Lys Ala Ile Trp Pro Ser Leu Gln Lys Ile Leu Thr Ser Val
1205 1210 1215

Tyr Thr Gly Gly Trp Asp Ile Asn Trp Lys Lys Tyr His Ala Pro Phe
1220 1225 1230

Ala Ser Ser Gln Lys Val Val Asp Leu Pro Ser Tyr Gly Trp Asp Leu
1235 1240 1245

Lys Asp Tyr Tyr Ile Pro Tyr Gln Gly Asp Trp Cys Leu His Arg His
1250 1255 1260

Gln Gln Asp Cys Lys Cys Ala Ala Pro Gly His Glu Ile Lys Thr Ala
1265 1270 1275 1280

Asp Tyr Gln Val Pro Pro Glu Ser Thr Pro His Arg Pro Ser Lys Leu
1285 1290 1295

Asp Pro Ser Lys Glu Ala Phe Pro Glu Ile Lys Thr Thr Thr Thr Leu
1300 1305 1310

His Arg Val Val Glu Glu Thr Thr Lys Pro Leu Gly Ala Thr Leu Val
1315 1320 1325

Val Glu Thr Asp Ile Ser Arg Lys Asp Val Asn Gly Leu Ala Arg Gly
1330 1335 1340

His Leu Val Asp Gly Ile Pro Leu Cys Thr Pro Ser Phe Tyr Ala Asp
1345 1350 1355 1360

Ile Ala Met Gln Val Gly Gln Tyr Ser Met Gln Arg Leu Arg Ala Gly
1365 1370 1375

His Pro Gly Ala Gly Ala Ile Asp Gly Leu Val Asp Val Ser Asp Met
1380 1385 1390

Val Val Asp Lys Ala Leu Val Pro His Gly Lys Gly Pro Gln Leu Leu
1395 1400 1405

Arg Thr Thr Leu Thr Met Glu Trp Pro Pro Lys Ala Ala Ala Thr Thr
1410 1415 1420

Arg Ser Ala Lys Val Lys Phe Ala Thr Tyr Phe Ala Asp Gly Lys Leu
1425 1430 1435 1440

Asp Thr Glu His Ala Ser Cys Thr Val Arg Phe Thr Ser Asp Ala Gln
1445 1450 1455

Leu Lys Ser Leu Arg Arg Ser Val Ser Glu Tyr Lys Thr His Ile Arg
1460 1465 1470

Gln Leu His Asp Gly His Ala Lys Gly Gln Phe Met Arg Tyr Asn Arg
1475 1480 1485

Lys Thr Gly Tyr Lys Leu Met Ser Ser Met Ala Arg Phe Asn Pro Asp
1490 1495 1500

Tyr Met Leu Leu Asp Tyr Leu Val Leu Asn Glu Ala Glu Asn Glu Ala
1505 1510 1515 1520

Ala Ser Gly Val Asp Phe Ser Leu Gly Ser Ser Glu Gly Thr Phe Ala
1525 1530 1535

Ala His Pro Ala His Val Asp Ala Ile Thr Gln Val Ala Gly Phe Ala
1540 1545 1550

Met Asn Ala Asn Asp Asn Val Asp Ile Glu Lys Gln Val Tyr Val Asn
1555 1560 1565

His Gly Trp Asp Ser Phe Gln Ile Tyr Gln Pro Leu Asp Asn Ser Lys
1570 1575 1580

Ser Tyr Gln Val Tyr Thr Lys Met Gly Gln Ala Lys Glu Asn Asp Leu
1585 1590 1595 1600

Val His Gly Asp Val Val Val Leu Asp Gly Glu Gln Ile Val Ala Phe
1605 1610 1615

Phe Arg Gly Leu Thr Leu Arg Ser Val Pro Arg Gly Ala Leu Arg Val
1620 1625 1630

Val Leu Gln Thr Thr Val Lys Lys Ala Asp Arg Gln Leu Gly Phe Lys
1635 1640 1645

Thr Met Pro Ser Pro Pro Pro Pro Thr Thr Thr Met Pro Ile Ser Pro
1650 1655 1660

Tyr Lys Pro Ala Asn Thr Gln Val Ser Ser Gln Ala Ile Pro Ala Glu
1665 1670 1675 1680

Ala Thr His Ser His Thr Pro Pro Gln Pro Lys His Ser Pro Val Pro
1685 1690 1695

Glu Thr Ala Gly Ser Ala Pro Ala Ala Lys Gly Val Gly Val Ser Asn
1700 1705 1710

Glu Lys Leu Asp Ala Val Met Arg Val Val Ser Glu Glu Ser Gly Ile
1715 1720 1725

Ala Leu Glu Glu Leu Thr Asp Asp Ser Asn Phe Ala Asp Met Gly Ile
1730 1735 1740

Asp Ser Leu Ser Ser Met Val Ile Gly Ser Arg Phe Arg Glu Asp Leu
1745 1750 1755 1760

Gly Leu Asp Leu Gly Pro Glu Phe Ser Leu Phe Ile Asp Cys Thr Thr
1765 1770 1775

Val Arg Ala Leu Lys Asp Phe Met Leu Gly Ser Gly Asp Ala Gly Ser
1780 1785 1790

Gly Ser Asn Val Glu Asp Pro Pro Pro Ser Ala Thr Pro Gly Ile Asn
1795 1800 1805

Pro Glu Thr Asp Trp Ser Ser Ser Ala Ser Asp Ser Ile Phe Ala Ser
1810 1815 1820

Glu Asp His Gly His Ser Ser Glu Ser Gly Ala Asp Thr Gly Ser Pro
1825 1830 1835 1840

Pro Ala Leu Asp Leu Lys Pro Tyr Cys Arg Pro Ser Thr Ser Val Val
1845 1850 1855

Leu Gln Gly Leu Pro Met Val Ala Arg Lys Thr Leu Phe Met Leu Pro
1860 1865 1870

Asp Gly Gly Gly Ser Ala Phe Ser Tyr Ala Ser Leu Pro Arg Leu Lys
1875 1880 1885

Ser Asp Thr Ala Val Val Gly Leu Asn Cys Pro Tyr Ala Arg Asp Pro
1890 1895 1900

Glu Asn Met Asn Cys Thr His Gly Ala Met Ile Glu Ser Phe Cys Asn
1905 1910 1915 1920

Glu Ile Arg Arg Arg Gln Pro Arg Gly Pro Tyr His Leu Gly Gly Trp
1925 1930 1935

Ser Ser Gly Gly Ala Phe Ala Tyr Val Val Ala Glu Ala Leu Val Asn
1940 1945 1950

Gln Gly Glu Glu Val His Ser Leu Ile Ile Ile Asp Ala Pro Ile Pro
1955 1960 1965

Gln Ala Met Glu Gln Leu Pro Arg Ala Phe Tyr Glu His Cys Asn Ser
1970 1975 1980

Ile Gly Leu Phe Ala Thr Gln Pro Gly Ala Ser Pro Asp Gly Ser Thr
1985 1990 1995 2000

Glu Pro Pro Ser Tyr Leu Ile Pro His Phe Thr Ala Val Val Asp Val
2005 2010 2015

Met Leu Asp Tyr Lys Leu Ala Pro Leu His Ala Arg Arg Met Pro Lys
2020 2025 2030

Val Gly Ile Val Trp Ala Ala Asp Thr Val Met Asp Glu Arg Asp Ala
2035 2040 2045

Pro Lys Met Lys Gly Met His Phe Met Ile Gln Lys Arg Thr Glu Phe
2050 2055 2060

Gly Pro Asp Gly Trp Asp Thr Ile Met Pro Gly Ala Ser Phe Asp Ile
2065 2070 2075 2080

Val Arg Ala Asp Gly Ala Asn His Phe Thr Leu Met Gln Lys Glu His
2085 2090 2095

Val Ser Ile Ile Ser Asp Leu Ile Asp Arg Val Met Ala
2100 2105

<210> 13
<211> 1986
<212> PRT
<213> Aspergillus nidulans

<400> 13
Met Glu Asp Pro Tyr Arg Val Tyr Leu Phe Gly Asp Gln Thr Gly Asp
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Phe Glu Val Gly Leu Arg Arg Leu Leu Gln Ala Lys Asn His Ser Leu
20 25 30

Leu Ser Ser Phe Leu Gln Arg Ser Tyr His Ala Val Arg Gln Glu Ile
35 40 45

Ser His Leu Pro Pro Ser Glu Arg Ser Thr Phe Pro Arg Phe Thr Ser
50 55 60

Ile Gly Asp Leu Leu Ala Arg His Cys Glu Ser Pro Gly Asn Pro Ala
65 70 75 80

Ile Glu Ser Val Leu Thr Cys Ile Tyr Gln Leu Gly Cys Phe Ile Asn
85 90 95

Tyr Tyr Gly Asp Leu Gly His Thr Phe Pro Ser His Ser Gln Ser Gln
100 105 110

Leu Val Gly Leu Cys Thr Gly Leu Leu Ser Cys Ala Ala Val Ser Cys
115 120 125

Ala Ser Asn Ile Gly Glu Leu Leu Lys Pro Ala Val Glu Val Val Val
130 135 140

Val Ala Leu Arg Leu Gly Leu Cys Val Tyr Arg Val Arg Lys Leu Phe
145 150 155 160

Gly Gln Asp Gln Ala Ala Pro Leu Ser Trp Ser Ala Leu Val Ser Gly
165 170 175

Leu Ser Glu Ser Glu Gly Thr Ser Leu Ile Asp Lys Phe Thr Arg Arg
180 185 190

Asn Val Ile Pro Pro Ser Ser Arg Pro Tyr Ile Ser Ala Val Cys Ala
195 200 205

Asn Thr Leu Thr Ile Ser Gly Pro Pro Val Val Leu Asn Gln Phe Leu
210 215 220

Asp Thr Phe Ile Ser Gly Lys Asn Lys Ala Val Met Val Pro Ile His
225 230 235 240

Gly Pro Phe His Ala Ser His Leu Tyr Glu Lys Arg Asp Val Glu Trp
245 250 255

Ile Leu Lys Ser Cys Asn Val Glu Thr Ile Arg Asn His Lys Pro Arg
260 265 270

Ile Pro Val Leu Ser Ser Asn Thr Gly Glu Leu Ile Val Val Glu Asn
275 280 285

Met Glu Gly Phe Leu Lys Ile Ala Leu Glu Glu Ile Leu Leu Arg Gln
290 295 300

Met Ser Trp Asp Lys Val Thr Asp Ser Cys Ile Ser Ile Leu Lys Ser
305 310 315 320

Val Gly Asp Asn Lys Pro Lys Lys Leu Leu Pro Ile Ser Ser Thr Ala
325 330 335

Thr Gln Ser Leu Phe Asn Ser Leu Lys Lys Ser Asn Leu Val Asn Ile
340 345 350

Glu Val Asp Gly Gly Ile Ser Asp Phe Ala Ala Glu Thr Gln Leu Val
355 360 365

Asn Gln Thr Gly Arg Ala Glu Leu Ser Lys Ile Ala Ile Ile Gly Met
370 375 380

Ser Gly Arg Phe Pro Glu Ala Asp Ser Pro Gln Asp Phe Trp Asn Leu
385 390 395 400

Leu Tyr Lys Gly Leu Asp Val His Arg Lys Val Pro Glu Asp Arg Trp
405 410 415

Asp Ala Asp Ala His Val Asp Leu Thr Gly Thr Ala Thr Asn Thr Ser
420 425 430

Lys Val Pro Tyr Gly Cys Trp Ile Arg Glu Pro Gly Leu Phe Asp Pro
435 440 445

Arg Phe Phe Asn Met Ser Pro Arg Glu Ala Leu Gln Ala Asp Pro Ala
450 455 460

Gln Arg Leu Ala Leu Leu Thr Ala Tyr Glu Ala Leu Glu Gly Ala Gly
465 470 475 480

Phe Val Pro Asp Ser Thr Pro Ser Thr Gln Arg Asp Arg Val Gly Ile
485 490 495

Phe Tyr Gly Met Thr Ser Asp Asp Tyr Arg Glu Val Asn Ser Gly Gln
500 505 510

Asp Ile Asp Thr Tyr Phe Ile Pro Gly Gly Asn Arg Ala Phe Thr Pro
515 520 525

Gly Arg Ile Asn Tyr Tyr Phe Lys Phe Ser Gly Pro Ser Val Ser Val
530 535 540

Asp Thr Ala Cys Ser Ser Ser Leu Ala Ala Ile His Leu Ala Cys Asn
545 550 555 560

Ser Ile Trp Arg Asn Asp Cys Asp Thr Ala Ile Thr Gly Gly Val Asn
565 570 575

Ile Leu Thr Asn Pro Asp Asn His Ala Gly Leu Asp Arg Gly His Phe
580 585 590

Leu Ser Arg Thr Gly Asn Cys Asn Thr Phe Asp Asp Gly Ala Asp Gly
595 600 605

Tyr Cys Arg Ala Asp Gly Val Gly Thr Val Val Leu Lys Arg Leu Glu
610 615 620

Asp Ala Leu Ala Asp Asn Asp Pro Ile Leu Gly Val Ile Asn Gly Ala
625 630 635 640

Tyr Thr Asn His Ser Ala Glu Ala Val Ser Ile Thr Arg Pro His Val
645 650 655

Gly Ala Gln Ala Phe Ile Phe Lys Lys Leu Leu Asn Glu Ala Asn Val
660 665 670

Asp Pro Lys Asn Ile Ser Tyr Ile Glu Met His Gly Thr Gly Thr Gln
675 680 685

Ala Gly Asp Ala Val Glu Met Gln Ser Val Leu Asp Val Phe Ala Pro
690 695 700

Asp His Arg Arg Gly Pro Gly Gln Ser Leu His Leu Gly Ser Ala Lys
705 710 715 720

Ser Asn Ile Gly His Gly Glu Ser Ala Ser Gly Val Thr Ser Leu Val
725 730 735

Lys Val Leu Leu Met Met Lys Glu Asn Met Ile Pro Pro His Cys Gly
740 745 750

Ile Lys Thr Lys Ile Asn His Asn Phe Pro Thr Asp Leu Ala Gln Arg
755 760 765

Asn Val His Ile Ala Leu Gln Pro Thr Ala Trp Asn Arg Pro Ser Phe
770 775 780

Gly Lys Arg Gln Ile Phe Leu Asn Asn Phe Ser Ala Ala Gly Gly Asn
785 790 795 800

Thr Ala Leu Leu Leu Glu Asp Gly Pro Val Ser Asp Pro Glu Gly Glu
805 810 815

Asp Lys Arg Arg Thr His Val Ile Thr Leu Ser Ala Arg Ser Gln Thr
820 825 830

Ala Leu Gln Asn Asn Ile Asp Ala Leu Cys Gln Tyr Ile Ser Glu Gln
835 840 845

Glu Lys Thr Phe Gly Val Lys Asp Ser Asn Ala Leu Pro Ser Leu Ala
850 855 860

Tyr Thr Thr Thr Ala Arg Arg Ile His His Pro Phe Arg Val Thr Ala
865 870 875 880

Ile Gly Ser Ser Phe Gln Glu Met Arg Asp Ser Leu Ile Ala Ser Ser
885 890 895

Arg Lys Glu Phe Val Ala Val Pro Ala Lys Thr Pro Gly Ile Gly Phe
900 905 910

Leu Phe Thr Gly Gln Gly Ala Gln Tyr Ala Ala Met Gly Lys Gln Leu
915 920 925

Tyr Glu Asp Cys Ser His Phe Arg Ser Ala Ile Glu His Leu Asp Cys
930 935 940

Ile Ser Gln Gly Gln Asp Leu Pro Ser Ile Leu Pro Leu Val Asp Gly
945 950 955 960

Ser Leu Pro Leu Ser Glu Leu Ser Pro Val Val Val Gln Leu Gly Thr
965 970 975

Thr Cys Val Gln Met Ala Leu Ser Ser Phe Trp Ala Ser Leu Gly Ile
980 985 990

Thr Pro Ser Phe Val Leu Gly His Ser Leu Gly Asp Phe Ala Ala Met
995 1000 1005

Asn Ala Ala Gly Val Leu Ser Thr Ser Asp Thr Ile Tyr Ala Cys Gly
1010 1015 1020

Arg Arg Ala Gln Leu Leu Thr Glu Arg Cys Gln Pro Gly Thr His Ala
1025 1030 1035 1040

Met Leu Ala Ile Lys Ala Pro Leu Val Glu Val Lys Gln Leu Leu Asn
1045 1050 1055

Glu Lys Val His Asp Met Ala Cys Ile Asn Ser Pro Ser Glu Thr Val
1060 1065 1070

Ile Ser Gly Pro Lys Ser Ser Ile Asp Glu Leu Ser Arg Ala Cys Ser
1075 1080 1085

Glu Lys Gly Leu Lys Ser Thr Ile Leu Thr Val Pro Tyr Ala Phe His
1090 1095 1100

Ser Ala Gln Val Glu Pro Ile Leu Glu Asp Leu Glu Lys Ala Leu Gln
1105 1110 1115 1120

Gly Ile Thr Phe Asn Lys Pro Ser Val Pro Phe Val Ser Ala Leu Leu
1125 1130 1135

Gly Glu Val Ile Thr Glu Ala Gly Ser Asn Ile Leu Asn Ala Glu Tyr
1140 1145 1150

Leu Val Arg His Cys Arg Glu Thr Val Asn Phe Leu Ser Ala Phe Glu
1155 1160 1165

Ala Val Arg Asn Ala Lys Leu Gly Gly Asp Gln Thr Leu Trp Leu Glu
1170 1175 1180

Val Gly Pro His Thr Val Cys Ser Gly Met Val Lys Ala Thr Leu Gly
1185 1190 1195 1200

Pro Gln Thr Thr Thr Met Ala Ser Leu Arg Arg Asp Glu Asp Thr Trp
1205 1210 1215

Lys Val Leu Ser Asn Ser Leu Ser Ser Leu Tyr Leu Ala Gly Val Asp
1220 1225 1230

Ile Asn Trp Lys Gln Tyr His Gln Asp Phe Ser Ser Ser His Arg Val
1235 1240 1245

Leu Pro Leu Pro Thr Tyr Lys Trp Asp Leu Lys Asn Tyr Trp Ile Pro
1250 1255 1260

Tyr Arg Asn Asn Phe Cys Leu Thr Lys Gly Ser Ser Met Ser Ala Ala
1265 1270 1275 1280

Ser Ala Ser Leu Gln Pro Thr Phe Leu Thr Thr Ser Ala Gln Arg Val
1285 1290 1295

Val Glu Ser Arg Asp Asp Gly Leu Thr Ala Thr Val Val Val His Asn
1300 1305 1310

Asp Ile Ala Asp Pro Asp Leu Asn Arg Val Ile Gln Gly His Lys Val
1315 1320 1325

Asn Gly Ala Ala Leu Cys Pro Ser Ser Leu Tyr Ala Asp Ser Ala Gln
1330 1335 1340

Thr Leu Ala Glu Tyr Leu Ile Glu Lys Tyr Lys Pro Glu Leu Lys Gly
1345 1350 1355 1360

Ser Gly Leu Asp Val Cys Asn Val Thr Val Pro Lys Pro Leu Ile Ala
1365 1370 1375

Lys Thr Gly Lys Glu Gln Phe Arg Ile Ser Ala Thr Ala Asn Trp Val
1380 1385 1390

Asp Lys His Val Ser Val Gln Val Phe Ser Val Thr Ala Glu Gly Lys
1395 1400 1405

Lys Leu Ile Asp His Ala His Cys Glu Val Lys Leu Phe Asp Cys Met
1410 1415 1420

Ala Ala Asp Leu Glu Trp Lys Arg Gly Ser Tyr Leu Val Lys Arg Ser
1425 1430 1435 1440

Ile Glu Leu Leu Glu Asn Ser Ala Val Lys Gly Asp Ala His Arg Leu
1445 1450 1455

Arg Arg Gly Met Val Tyr Lys Leu Phe Ser Ala Leu Val Asp Tyr Asp
1460 1465 1470

Glu Asn Tyr Gln Ser Ile Arg Glu Val Ile Leu Asp Ser Glu His His
1475 1480 1485

Glu Ala Thr Ala Leu Val Lys Phe Gln Ala Pro Gln Ala Asn Phe His
1490 1495 1500

Arg Asn Pro Tyr Trp Ile Asp Ser Phe Gly His Leu Ser Gly Phe Ile
1505 1510 1515 1520

Met Asn Ala Ser Asp Gly Thr Asp Ser Lys Ser Gln Val Phe Val Asn
1525 1530 1535

His Gly Trp Asp Ser Met Arg Cys Leu Lys Lys Phe Ser Ala Asp Val
1540 1545 1550

Thr Tyr Arg Thr Tyr Val Arg Met Gln Pro Trp Arg Asp Ser Ile Trp
1555 1560 1565

Ala Gly Asn Val Tyr Ile Phe Glu Gly Asp Asp Ile Ile Ala Val Phe
1570 1575 1580

Gly Gly Val Lys Phe Gln Ala Leu Ser Arg Lys Ile Leu Asp Ile Ala
1585 1590 1595 1600

Leu Pro Pro Ala Gly Leu Ser Lys Ala Gln Thr Ser Pro Ile Gln Ser
1605 1610 1615

Ser Ala Pro Gln Lys Pro Ile Glu Thr Ala Lys Pro Thr Ser Arg Pro
1620 1625 1630

Ala Pro Pro Val Thr Met Lys Ser Phe Val Lys Lys Ser Ala Gly Pro
1635 1640 1645

Ser Val Val Val Arg Ala Leu Asn Ile Leu Ala Ser Glu Val Gly Leu
1650 1655 1660

Ser Glu Ser Asp Met Ser Asp Asp Leu Val Phe Ala Asp Tyr Gly Val
1665 1670 1675 1680

Asp Ser Leu Leu Ser Leu Thr Val Thr Gly Lys Tyr Arg Glu Glu Leu
1685 1690 1695

Asn Leu Asp Met Asp Ser Ser Val Phe Ile Glu His Pro Thr Val Gly
1700 1705 1710

Asp Phe Lys Arg Phe Val Thr Gln Leu Ser Pro Ser Val Ala Ser Asp
1715 1720 1725

Ser Ser Ser Thr Asp Arg Glu Ser Glu Tyr Ser Phe Asn Gly Asp Ser
1730 1735 1740

Cys Ser Gly Leu Ser Ser Pro Ala Ser Pro Gly Thr Val Ser Pro Pro
1745 1750 1755 1760

Asn Glu Lys Val Ile Gln Ile His Glu Asn Gly Thr Met Lys Glu Ile
1765 1770 1775

Arg Ala Ile Ile Ala Asp Glu Ile Gly Val Ser Ala Asp Glu Ile Lys
1780 1785 1790

Ser Asp Glu Asn Leu Asn Glu Leu Gly Met Asp Ser Leu Leu Ser Leu
1795 1800 1805

Thr Val Leu Gly Lys Ile Arg Glu Ser Leu Asp Met Asp Leu Pro Gly
1810 1815 1820

Glu Phe Phe Ile Glu Asn Gln Thr Leu Asp Gln Ile Glu Thr Ala Leu
1825 1830 1835 1840

Asp Leu Lys Pro Lys Ala Val Pro Thr Ala Val Pro Gln Ser Gln Pro
1845 1850 1855

Ile Thr Leu Pro Gln Ser Gln Ser Thr Lys Gln Leu Ser Thr Arg Pro
1860 1865 1870

Thr Ser Ser Ser Asp Asn His Pro Pro Ala Thr Ser Ile Leu Leu Gln
1875 1880 1885

Gly Asn Pro Arg Thr Ala Ser Lys Thr Leu Phe Leu Phe Pro Asp Gly
1890 1895 1900

Ser Gly Ser Ala Thr Ser Tyr Ala Thr Ile Pro Gly Val Ser Pro Asn
1905 1910 1915 1920

Val Ala Val Tyr Gly Leu Asn Cys Pro Tyr Met Lys Ala Pro Glu Lys
1925 1930 1935

Leu Thr Cys Ser Leu Asp Ser Leu Thr Thr Pro Tyr Leu Ala Glu Ile
1940 1945 1950

Arg Arg Arg Gln Pro Thr Gly Pro Tyr Asn Leu Gly Gly Trp Ser Gln
1955 1960 1965

Ala Gly Ser Ala His Thr Thr Arg His Ala Ser Ser Tyr Cys Ser Arg
1970 1975 1980

Ala Lys
1985

<210> 14
<211> 53
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

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atgaagcttg gggtttgagg gccaatggaa cgaaactagt gtaccacttg acc 53

<210> 15
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 15
gacagatctg gcgccattcg ccattcag 28

<210> 16
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 16
ggaatcggtc aatacactac 20

<210> 17
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 17
tgtagatctc tattcctttg ccctcggacg agt 33

<210> 18
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 18
ggccgccacg gatatcttgg ccaaagaatt cctgg 35

<210> 19
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 19
cggtgcctat agaaccggtt tcttaaggac cgcgc 35

<210> 20
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 20
gayccmgtty ttyaayatg 19

<210> 21
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 21
gtccgtccrt gcatytc 17

<210> 22
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 22
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<223> Description of Artificial Sequence: Primer

<400> 23
aaatggcgcg ccgcgcccag aatgacacc 29

<210> 24
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<400> 24
tgccacctgt agtctgcaat cag 23

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ctacatcgag atgcacggca c 21

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ngtcgaswga nawgaa 16

<210> 29
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gtncgaswca nawgtt 16

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<400> 30
wgtgnagwan canaga 16

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cawcgcngaa sgaa 14

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<400> 37
tgagacagat ctcgcgagcc ctc 23

<210> 38
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<400> 38
atgtctccaa aggaagctga gc 22

<210> 39
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<400> 39
tcgagtgatg gatactgctt cg 22

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<210> 46
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<210> 48
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<210> 49
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<220>
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<400> 49
ctttgatctt ttctacgggg tctga 25